

# Philip Siekevitz: Bridging biochemistry and cell biology

Philip Siekevitz, an Emeritus Professor at the Rockefeller University who made pioneering contributions to the development of modern cell biology, passed away on December 5th, 2009. He was a creative and enthusiastic scientist, as well as a great experimentalist who throughout his lifetime transmitted the joy of practicing science and the happiness that comes with the acquisition of new knowledge. He was a man of great integrity, with a thoroughly engaging personality and a humility not often found in people of his talent.

Philip Siekevitz's career proceeded along three phases marked by seminal contributions that opened up new avenues of research. The first phase was in the field of protein synthesis, in which he developed the first *in vitro* system using defined cell fractions. Then, in collaboration with George Palade, he demonstrated the central role of ribosomes in protein synthesis and, in particular, of membrane-bound ribosomes in the synthesis of secretory proteins. In later stages of his career Siekevitz turned his attention to the nervous system, where he isolated and characterized the postsynaptic density, a structure that integrates the activity of many transmembrane and associated proteins in mediating synaptic transmission.

## Early years

Siekevitz was born in 1918 in South Philadelphia, where his immigrant father was a skilled worker in a garment factory and his mother was a dressmaker. His interest in biology began in high school, but he was a child of the Depression and upon graduation spent two years working to earn the funds necessary to pay for a college education. He attended the Philadelphia College of Pharmacy and Science, where he developed an interest in biochemistry. However, soon after graduation, in 1942, he entered the Army and served in a decontamination unit prepared to respond to chemical warfare

attacks. Because he was eager to enhance his scientific background, Siekevitz requested a transfer, which resulted in his deployment as a laboratory technician to an Air Force Supply Base for the Pacific War in San Bernardino, California, where he honed his skills in microscopy and chemical analysis.

In 1945, after three and a half years in the service, Siekevitz was admitted to the graduate program of the already highly regarded Biochemistry Department of the University of California, then at Berkeley, where his tuition and living expenses were provided by the GI Bill.

David Greenberg, head of the program and Phil's PhD thesis advisor, recalled later that the department had gradually contracted during the war years but, with peace, turmoil descended upon it in the form of a huge influx of beginners all of whom, like Phil, had hiatuses in their careers. Phil was attracted to the mysteries of protein synthesis, but Greenberg steered him to the study of the metabolism of the amino acids glycine and serine in liver slices, for which Siekevitz took advantage of the availability of  $^{14}\text{C}$  produced in a nearby cyclotron.

## Protein synthesis *in vitro*

In 1949 Phil turned 31, received his PhD, and married Rebecca Burstein, who remained his wife for 60 years. The Siekevitzes then moved to Boston, where Phil, with a public health service fellowship, joined the group of Paul Zamecnik at the Huntington Laboratories of Harvard University at the Massachusetts General Hospital. Zamecnik was a major contributor to the elucidation of the molecular events in protein synthesis. Siekevitz threw himself with enthusiasm into this field at a time in which only three laboratories in the country were beginning to explore its biochemical mysteries.

Up to then, most work on protein synthesis had been performed by measuring the incorporation of radioactive

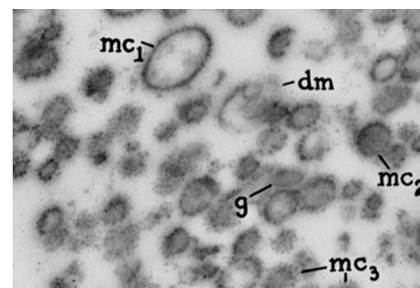
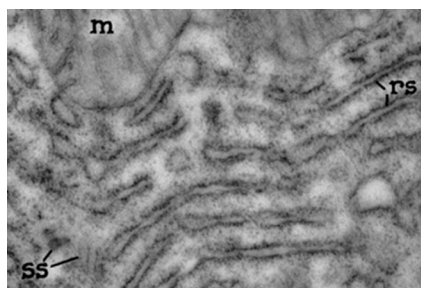


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amino acids into proteins in whole animals or in tissue slices incubated *in vitro* because acellular homogenates had shown minimal activity. Siekevitz achieved a breakthrough by taking advantage of cell fractionation techniques. In later work at the Rockefeller Institute, he developed this technique to new heights and called it "a bridge between the morphologists and the biochemists." In a collaboration with Zamecnik, and in an impressive and comprehensive paper (Siekevitz, 1952) of which he was the sole author, Siekevitz first related biochemical requirements to recognizable structures. He examined the protein-synthesizing activity of an *in vitro* system in which he combined in various ratios mitochondrial, microsomal, and supernatant-derived fractions. Using this approach, he demonstrated the synergistic role of energy-producing mitochondria in sustaining the activity of microsomes, which showed the highest rate of incorporation of labeled amino acids into their proteins. In Siekevitz's system mitochondria were fueled by  $\alpha$ -ketoglutarate or succinate, which sustained the production by the mitochondria of a soluble factor that enabled the microsomes to incorporate alanine into its proteins. Because Siekevitz showed that the factor was consumed when hexokinase was added to the system, he

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Figure 1. **Microsomes are fragments of the rough ER.** (Left) Electron micrograph showing the cytoplasm in an acinar cell of the guinea pig pancreas. rs, rough ER; ss, smooth ER; m, mitochondria. (Right) An EM section of a microsome pellet isolated from pancreatic cells. Note the presence of various kinds of membrane-bound vesicles ( $mc_1$ ,  $mc_2$ , and  $mc_3$ ), most of which are studded with dense particles that were later identified to be ribosomes. dm, damaged mitochondria; g, dense granules. From Palade and Siekevitz (1956b).



suggested that the factor could be ATP, the universal energy currency discovered by Fritz Lippman.

Phil's system was the forerunner of many other advances in Zamecnik's laboratory, and in other laboratories, which elucidated the mechanism of amino acid activation (Hoagland et al., 1956). Two years after Siekevitz's paper, Zamecnik and Elizabeth Keller (Zamecnik and Keller, 1954) introduced a major simplification by replacing the mitochondria with a soluble ATP-generating system. Further biochemical dissection in Zamecnik's laboratory of the supernatant-derived fraction led to the momentous discovery of tRNA (Hoagland et al., 1958).

Siekevitz's discovery of the role of the mitochondrion as the energy supplier for protein synthesis led him to apply for an oncology fellowship to work with Van Potter, then a pioneer of research on energy metabolism, at the McArdle Cancer Laboratories of the University of Wisconsin in Madison, the campus where Phil's wife, Rebecca, had been a student. It was during this fellowship that their two daughters, Ruth and Miriam, were born.

Siekevitz's time with Van Potter (1951–1954) was highly productive, with him publishing six papers on the enzymology and compartmentalization of adenine nucleotide metabolism, all but one of them in *The Journal of Biological Chemistry*.

### **The central biosynthetic role of the ER**

Keith Porter and George Palade, who at the Rockefeller Institute were making trailblazing discoveries with the electron microscope on the identification and structure of subcellular organelles, were among the many who appreciated Siekevitz's development of an *in vitro* protein-synthesizing system. In fact,

Porter already cited Siekevitz in a 1953 paper in *The Journal of Experimental Medicine* (Porter, 1953), and in 1954, Palade invited Siekevitz to present his work at the Rockefeller Institute. During this visit Siekevitz was easily persuaded to join Palade's group.

Together, Siekevitz and Palade undertook what has been referred to as “the chemical dissection of the microsomes,” as well as the elucidation of the function of the small particles that Palade had observed in the electron microscope either free in the cytoplasm or bound to the cisternal membranes of the rough portions of the endoplasmic reticulum (ER; Palade, 1955). In a masterful combination of cell fractionation, biochemistry, and electron microscopy (Palade and Siekevitz, 1956a,b), the team established that microsomes arise by a peculiar fragmentation of the ER during which membrane vesicles with their attached particles pinch off from ER cisterna without leakage of the cisternal content (Fig. 1). In a study of liver mitochondria that Siekevitz was carrying out with Michael Watson, he had used sodium deoxycholate to solubilize mitochondrial membranes and found that this detergent also effectively solubilized microsomal membranes—allowing for the recovery of the attached particles by high-speed centrifugation. The particles were shown to be rich in protein and RNA, and hence, were named RNPs for ribonucleoprotein particles.

Although the initial work on microsomes was performed in the liver, Palade and Siekevitz chose the guinea pig pancreas for their subsequent work because Palade had recognized the extraordinary development of the ER in the acinar cells of this organ, which manufactures prodigious amounts of digestive enzymes. It was with this system that in subsequent seminal papers Siekevitz and Palade

showed that the membrane-bound RNPs, later named ribosomes, were the exclusive site of synthesis of pancreatic enzymes (Siekevitz and Palade, 1958b,c).

Palade's and Siekevitz's further studies focused on the kinetics of labeling of secretory proteins recovered in different subcellular fractions after labeling *in vivo* with injected radioactive amino acids (Siekevitz and Palade, 1958a, 1959, 1960). This allowed them to show that pancreatic enzymes synthesized in membrane-bound ribosomes—in *vivo*, as well as *in vitro* with incubated microsomes (Redman et al., 1966)—subsequently accumulate in the lumen of the microsomal vesicles from where they could be released after membrane solubilization. This work laid the foundation for the subsequent studies by Jim Jamieson and Palade that traced the pathway of newly synthesized pancreatic enzyme precursors from the ER to the Golgi apparatus, where they are concentrated in zymogen granules to be discharged at the cell surface.

At Rockefeller, Siekevitz developed a strong interest in membrane biochemistry and, in particular, in organellar membrane biogenesis. In following years he and the young scientists who joined his laboratory played a major role in studies of the biosynthesis, structure, and function of the ER, chloroplast, and neuronal membrane proteins.

Siekevitz's work on the postsynaptic density (PSD), which began in the mid-1970s—like his earlier research on the protein-synthesizing apparatus of secretory cells—was driven by his ability to devise approaches to isolate subcellular structures first identified *in situ* by electron microscopy and to characterize them biochemically. The postsynaptic density had been visualized in the 1950s, and Siekevitz and co-workers refined existing

procedures for their isolation by detergent treatment of synaptosomal fractions. This allowed his group to identify within the PSD signaling molecules and ion channels involved in nerve impulse transmission (for example, see Wu et al., 1985, 1992). It is now recognized that the PSD represents a macromolecular assembly that organizes the postsynaptic signaling machinery. In a theoretical paper (Siekevitz, 1985), Siekevitz presciently postulated that long-lasting changes in neuronal circuitry result from “changes in the concentration and conformation of PSD proteins, changes that could alter the neurophysiology of dendritic spines.”

### A man with a roving intellect and deep ethical convictions

Siekevitz had many admirable personal attributes that endeared him to those in the biochemistry and cell biology communities with whom he interacted and, in particular, to those, like myself, whom he hosted in his laboratory and very generously advised and supported without expecting recognition of his influence or his contributions.

Siekevitz was also a highly principled person who adhered to the highest standards of professional and personal behavior. His publications are shining examples of how he went out of his way to give due credit to others who might have preceded him with discoveries in the field of his work.

Siekevitz’s concern for ethical issues impinging on the behavior of scientists led him to often write articles and letters to the Editor in journals and newspapers, ranging from *Nature*, *Science*, *The Scientist*, and *The Nation* to *The New York Times*. He commented on issues related to science and society, including the hubris of scientists who neglected their responsibility to inform the public of the implications of their research. He was particularly concerned that in the era of rapid biotechnological advances, scientists were tempted to profit inordinately from discoveries that were made with public funds. He regretted that “the mixture of science and money” was poisoning the atmosphere of free inquiry and disinterested cooperation in which science thrives best. He also feared that competition for important scientific prizes

was fostering secrecy and preventing due recognition of the research achievements made by others working in the same area. These grave concerns did not diminish Siekevitz’s amiable collegiality or his willingness to generously give his time to others and to share his knowledge, as much with important peers as with younger beginners who sought his advice or counsel.

Siekevitz had a fertile mind and an insatiable and roving intellectual curiosity. He did not limit his intellectual pursuits to the biological sciences, but read avidly in the physical and social sciences. Beyond the over 120 papers that reported his scientific contributions, he found time to write with Ariel Loewy in 1963, “Cell Structure and Function,” the first American textbook in cell biology, which underwent two more editions. He had literary talents and published in New Directions Press two fictional novellas, “The Petition” in 1948, and “The Fish” in 1950. He played the piano, with Mozart and Beethoven being his favorites, and in 1988 began to write a series of fictional short stories on Mozart, his family, his operas, and their characters, all of them yet unpublished.

For his scientific achievements, Siekevitz gathered too many honors to be listed here. He was President of the American Society for Cell Biology in 1966, being preceded by Van Potter and George Palade, and of the New York Academy of Sciences. He was elected to the National Academy of Sciences in 1975 and chaired the section on cellular and developmental biology. He was a fellow of the American Academy of Arts and Sciences and of the American Association for the Advancement of Science. He received honorary degrees from his college Alma Mater and from the University of Stockholm in 1974. He was the editor of the *JCB* from 1961 to 1964 and served in editorial boards of many other journals.

David Sabatini  
david.sabatini@nyumc.org

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