

## Har Gobind Khorana (1922–2011): Chemical Biology Pioneer

Thomas P. Sakmar\*

Laboratory of Molecular Biology and Biochemistry, The Rockefeller University, 1230 York Avenue, New York, New York 10065, United States

H. Gobind Khorana, a visionary chemist and a founder and pioneer of what we now call chemical biology, died on November 9, 2011. He was 89.

Gobind was an innovative chemist who spent a 50-plus-year career applying multidisciplinary approaches to solve problems at the boundaries between chemistry and molecular biology. He made major contributions to several fields and devised numerous methods that we now take for granted, including gene synthesis, solid-phase oligonucleotide synthesis, and polymerase chain reaction. Despite an unassuming demeanor and modest character, Gobind's drive, perseverance, and work ethic were legendary.

For his work on the elucidation of the genetic code, which was one of the great scientific achievements of the age of molecular biology, he shared the Nobel Prize for Physiology or Medicine in 1968 (with Robert W. Holley and Marshall W. Nirenberg). Gobind's group at the Institute for Enzyme Research at the University of Wisconsin at Madison worked around the clock in double shifts to synthesize all of the possible triplet trinucleotides, thus providing a firm basis to establish the complete codon assignments and to determine how the code was read.

Although Gobind came under the influence of a roster of scientific luminaries during his early training, including Alexander R. (Lord) Todd at University of Cambridge and Vladimir Prelog at Eidgenössische Technische Hochschule in Zurich, the real turning point in his career came in 1954 at the British Columbia Research Council, Vancouver, with his sole-author publication of the chemical synthesis of ADP and ATP using the carbodiimide reaction.<sup>1</sup>

In the 1940s, if you wanted a sample of pure ATP, you would begin your preparation with a live rabbit—seriously.<sup>2</sup> Gobind later synthesized, with the help of several notable colleagues (Gordon W. Tener, John G. Moffat, Michael Smith, and others) every significant known nucleotide and nucleotide co-factor. The culmination of this phase of his career was the synthesis of coenzyme A, by far the most complex of the nucleotide co-factors, in 1960.<sup>3</sup>

I remember a conversation that I had with Gobind in the late 1980s at the Massachusetts Institute of Technology. We were discussing the rationale and wisdom of the term “chemical biology”. Was chemical biology a discrete field? When did it begin? Should the venerable Department of Chemistry at Harvard University change its name? (The name did eventually change to the Department of Chemistry and Chemical Biology, which certainly does not clarify the matter very much except to suggest that the two fields are distinct.) Gobind listened intently to my point of view, then he leapt up from his chair and bound toward a filing cabinet. Within seconds he pulled out a reprint. His files were almost exclusively organized by scientists' names—no need for keywords or alphabetical subject



H. G. Khorana, 1969. Credit: Hulton Archive/Getty Images.

lists. “Here is when chemical biology began,” he said, “January 5, 1955.... Read this!”<sup>4</sup>

The story of how Eugene P. Kennedy and his graduate student Samuel B. Weiss discovered that cytidine diphosphate (CDP) choline was the intermediate in phosphatidylcholine synthesis is well-known to many. Kennedy heard about Gobind's 1954 paper using the carbodiimide reaction from Fritz Lipmann and immediately went to work to synthesize CDP-choline using the “elegant procedure of Khorana”.

However, what is not so well-known is that according to Gobind, the work of Kennedy provided a great catalyst that promoted the general awareness of Gobind's chemical methods among the biochemical community. Up until that time, chemistry was relegated to confirm the structures of biological molecules that had been isolated from biological samples and then analyzed, usually by degradation. Synthetic versions were prepared and then tested to confirm their structure and for biological activity. In the case of Kennedy's work, we have the entirely different situation in which samples were prepared by chemical synthesis and tested even before they had been isolated from natural sources.

Suddenly everyone wanted to know how to make nucleotides of potential biological interest. During summer “vacations” notable scientists including Paul Berg, Arthur Kornberg, Leon Heppel, Saul Roseman, Irving Goldberg, Herman M. Kalckar, Rollin Hotchkiss, and others visited Gobind's laboratory to learn how to prepare and use the new carbodiimide reagents.

The course of Gobind's career was greatly influenced by his contact with these great biochemists at the dawn of the age of molecular biology. His line of thinking was clearly outlined in 1960 with a provocative exhortation that should cement his designation as the father of chemical biology.<sup>5</sup>

Published: February 17, 2012

"In the distant future, the total chemical synthesis of macromolecules possessing biological function must also be considered. The problems are vastly more complex than anything previously undertaken by experimental organic chemistry. Nevertheless, Biology urgently asks: Will Organic Chemistry extend its horizons and accept the challenge?"

And of course, this statement was rhetorical because Gobind had already decided to move forward with plans to synthesize a functional gene. At the time only dinucleotides could be made in satisfactory yield, and there was no reliable method to sequence DNA. So most people who were aware of Gobind's plans at the time were probably shaking their heads dismissively, but of course we now have the luxury of knowing that Gobind succeeded.

In 1972 Gobind's team, now at the Massachusetts Institute of Technology, described a monumental achievement in chemical biology: the total chemical synthesis of a functional tRNA gene, which was published in an entire issue of *Journal of Molecular Biology* in December 1972, 15 consecutive articles, 313 consecutive pages.

Gobind's remarkable scientific achievements resulted, at least in part, from his remarkable intellectual agility. He walked a figurative tightrope between scientific cultures and disciplines. He had the audacity to make major contributions to emerging fields and then to move on to other interests and projects. He was a true innovator, although his base of operations never really extended beyond the laboratory. He left a remarkable trail of technical achievements, some of which were truly transformative, as he continued to reinvent himself at regular intervals for more than 50 years. He thought constantly about effort and commitment, sometimes doubling down when projects seemed stalled. But he also seemed to know when to quit, or better stated, when to move on to other subjects, sometimes related, but often totally new.

One example is when he switched from working on polynucleotides and gene synthesis and took up the subject of membrane proteins in the mid-1970s. Within about 5 years, he would publish the complete amino acid sequence of bacteriorhodopsin from purple membrane, the first integral membrane protein to be sequenced. He then cloned the gene, worked out a heterologous expression scheme, and used site-directed mutagenesis (just invented by his former colleague M. Smith) to elucidate mechanism. Related work on the G protein-coupled receptor, rhodopsin, went on essentially in parallel, and many of the methods pioneered by Gobind's group, including the use of immunoaffinity purification, were used later to advance the structural biology of GPCRs.

But when I think now about Gobind, what I miss most, what I grieve over most deeply, is not so much the loss of a great scientist as the loss of such a remarkable man with such an amazing life story. How is it possible? How is it possible that the son of tax clerk from an impoverished village in Punjab, who as a boy would sit on the steps of the post office and transcribe letters for illiterate townspeople, would as a young man ride away on an elephant on the first leg of a journey that would lead him to England, Switzerland, Canada, and America?<sup>6</sup> Gobind's life journey would bring him in contact with royalty, presidents, and prime ministers, with famous scientists and intellectuals as wide ranging as Leo Szilard and Bertrand Russell.

Yet Gobind always maintained that sense of focus and commitment to his laboratory work and his dedication to his colleagues, perhaps 250 in all during the period 1954 to 2007.

In my own case, while I worked with Gobind at MIT from July 1985 through August 1990, I overlapped with 36 other graduate students and postdoctoral fellows. As a member of Gobind's group in the late 1980s, I saw a man still totally focused on laboratory work, still following an almost relentless schedule, three or four group meetings a week, plus Saturday tea. Most of the meetings were shared jointly with the laboratory of Uttam L. RajBhandary, who as a long-term colleague and constant presence at M.I.T. contributed immeasurably to Gobind's success and longevity.

H. Gobind Khorana, the great chemist and innovator, traveled one of the most remarkable personal and scientific journeys of the 20th century. With Gobind's death, I feel an immense sense of loss, and I grieve the passing of a truly great man.

## AUTHOR INFORMATION

### Corresponding Author

\*E-mail: sakmar@rockefeller.edu.

## REFERENCES

- (1) Khorana, H. G. (1954) Carbodiimides. Part V. A novel synthesis of adenosine di- and triphosphate and P<sup>1</sup>,P<sup>2</sup> diadenosine-5'-pyrophosphate. *J. Am. Chem. Soc.* 76, 3517–3522.
- (2) Kerr, S. E. (1941) On the preparation of adenosine triphosphate. *J. Biol. Chem.* 139, 121–130.
- (3) Moffat, J. G., and Khorana, H. G. (1961) Nucleoside polyphosphates. XII. The total synthesis of coenzyme A<sup>2</sup>. *J. Am. Chem. Soc.* 83, 663–675.
- (4) Kennedy, E. P., and Weiss, S. B. (1955) Cytidine diphosphate choline: A new intermediate in lecithin biosynthesis. *J. Am. Chem. Soc.* 77, 250–251.
- (5) Khorana, H. G. (1960) Synthesis of nucleotides, nucleotide coenzymes and polynucleotides. *Fed. Proc.* 19, 931–941.
- (6) For additional details about H.G.K.'s early life and career, see: Sakmar, T. P. (2012) Har Gobind Khorana (1922–2011): Pioneering Spirit. *PLoS Biology*, 10 (2): e1001273. doi:10.1371/journal.pbio.1001273.