HAR GOBIND KHORANA

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9 JANUARY 1922 · 9 NOVEMBER 2011

Har Gobind Khorana was one of the founding spirits of what we now call chemical biology and a pioneer at the dawn of the molecular biology era. Gobind traveled an almost unfathomable journey from an impoverished village in Punjab in British India to become one of the most important scientists of the molecular biology era. His work on chemical synthesis of polynucleotides, the building blocks of DNA, led to the elucidation of the genetic code and to a share of the Nobel Prize in Physiology or Medicine in 1968 with Robert W. Holley and Marshall W. Nirenberg.

Age 46 at the time of the Nobel ceremony, Gobind had already contributed landmark achievements during the golden age of molecular biology; he then went on to report the first chemical synthesis of a functional gene just four years later. Subsequently, in a surprising (to some) transformation, he began nearly three decades of work on membrane proteins, particularly the light-driven proton pump, bacteriorhodopsin, and the visual pigment, rhodopsin. He made many groundbreaking contributions in applying chemical and molecular methods to studies of membrane proteins and championed studies of rhodopsin as a model for transmembrane signal transduction.

There are many apocryphal stories about Gobind's early education and later scientific training, but most of what I know about the man came directly from conversations over a 25-year period of friendship, and through close interactions with many of his loyal and devoted colleagues. The level of respect, admiration, and affection that Gobind's colleagues felt for him was remarkable, and he worked with and influenced a notable group of scientists over the years. They all have their stories to tell. Gobind himself was modest and a bit reticent, but at key points in his career, he did write autobiographic chapters. And on his retirement he authored a multi-chapter book with reprints of his key publications, entitled *Chemical Biology*, which includes introductory personal perspectives for each chapter.

But details about Gobind's early life still remain somewhat of a mystery. His ancestral village of 100 or so families in rural Punjab would end up in Pakistan after the Partition of 1947 and most of his immediate family would migrate to India. He would not return to his original home after 1945. Gobind's father, a local patwari—or land registrar—taught him to read as a young child and helped to establish a single-room school. As a young child, Gobind woke up early to go out into the village, look for a house with smoke coming out of the chimney, and then ask for a bit of ember to take home to light the cooking fire. He would later sit on the steps of a nearby post office and transcribe letters for townspeople, most of whom were illiterate. It was there that he undoubtedly developed his characteristic micro-script handwriting, which anyone who ever worked with him on a manuscript draft would instantly recognize. It was there that he also developed his trademarks of frugality—using lead pencils until they were barely long enough to grip, and taking notes on any scrap of paper that happened to be available. Of course, his actual laboratory notebooks were models of clarity and organization.

Whether he actually rode away from his village on an elephant to leave for university is not entirely clear (he told me that he did, but with a bit of a wry smile). What is maybe not so well known is that he had a special affection for the work of George Bernard Shaw and claims to have traveled to Lahore with the intention of studying English literature. Apparently, though, when Gobind arrived at university, he found out that the matriculation examination for literature included an oral component, which he was too shy to complete. Can it be possible that chemistry was really the default curriculum for this master bioorganic chemist? In any case, he received an M.Sc. degree in chemistry from Punjab University in 1945.

Gobind also outlined how he ended up in postwar Liverpool, England in 1945. India, as the world's fourth-largest industrial economy at the end of the war, was preparing for independence and modernization and initiated an ambitious plan to send students abroad temporarily for scientific and technical training. Gobind was a part of the first cohort of students selected by the Ministry of Agriculture. He was assigned to an institute in Berkshire, England to study insecticides and fungicides for agriculture development. But by the time Gobind had reported to the Indian High Commissioner in London, the position in Berkshire had disappeared, so he was assigned without his prior knowledge directly to a Ph.D. program in the Department of Organic Chemistry at Liverpool University—a fortuitous misdirection that would come to characterize Gobind's travels for the subsequent 25 years.

Gobind was assigned to the laboratory of Roger J. S. Beer, who had just joined the faculty. Not only did Beer work side by side with Gobind on chemical studies of alkaloid synthesis and melanin, but he also took a personal interest in Gobind's life and career. When Gobind completed his dissertation in the spring of 1948, he was supposed to return to India to fulfill the requirements of his earlier scholarship, but Gobind was not ready to return. He wrote that he wanted "to spend a period of time in a laboratory in a German-speaking region of Europe." But since the only practical option at the time would have been Switzerland, I suspect that his official explanation is only a part of the story. In fact, earlier in the summer of 1947, Gobind had traveled to Prague for the first World Festival of Youth and Students. It was there in a crowd at a rally that somehow Gobind first met Esther Silber, a young Swiss woman. They exchanged addresses and began to write to each other. Gobind applied to the Indian government for a postdoctoral fellowship to spend the next year in Switzerland, but was turned down. He moved to Switzerland anyway with the ostensible aim of finding a suitable laboratory position.

The relatively short time Gobind spent at Eidgenössische Technische Hochschule in Zurich in 1948 with Vladimir Prelog was the most important, and probably most difficult, period in his life. He came to Zurich without a formal letter of introduction or reference and just walked in on Prelog. Based on a quick review of his dissertation, which Gobind eagerly presented, Prelog accepted him as a postdoctoral fellow, but still no funding was forthcoming. Gobind essentially lived in the laboratory on rice and unpasteurized milk for the next 11 months (official biographies say that he subsisted on "savings"). Despite the hardships, Gobind formed an instant and long-lasting bond with Prelog, a legendary mentor whom Gobind credits for influencing his approach to work and his integrity as a scientist. Prelog would go on to share the Nobel Prize in Chemistry in 1975 for his contributions to stereochemical principles and practice in organic synthesis.

Ironically, it was Gobind's initial lack of progress at the bench that serendipitously led to his eventual spectacular success. He spent hours in the library reviewing the German organic chemistry literature, and came across a description of a little-known synthetic reagent, carbodiimide, that had been essentially forgotten and never surfaced in the English literature. Though of no use to his work at the time, Gobind would remember the reagent years later and apply it to create a revolution in biochemistry.

In 1949, Gobind was required to return to India to fulfill the requirements of service mandated by his earlier scholarship, but in post-partition India his ancestral village had ended up in Pakistan and his family had dispersed. Unable to find work and living in the servants' quarters of his uncle's house in New Delhi, Gobind became essentially an academic refugee, spending fruitless months looking for work. Thankfully, the government annulled the bond to repay his scholarship and he accepted a fellowship to work with Alexander R. Todd at Cambridge, thanks to the help of Cambridge professor G. W. Kenner, whom Gobind had met and worked with in Zurich. Lord Todd went on to win the Nobel Prize in Chemistry in 1957 for studies on nucleosides and nucleoside cofactors.

In late 1949 at age 27, Gobind returned to England after his extended family scraped together the fare for a ship's passage. Kenner and Khorana were reunited in the laboratory and initially used carbodiimide reagents to activate the carboxyl-terminal ends of peptides. But Todd had just deciphered the correct chemical linkages between nucleotides in DNA, and Gobind was attracted to the new field of molecular biology.

Gobind also was reunited in Cambridge with Esther Silber. As luck would have it, Gordon M. Shrum, head of the British Columbia Research Council of Canada, visited Cambridge and asked Todd to suggest a chemist who might be willing to move to Vancouver to start a new nonacademic research laboratory—no start-up package, but unlimited freedom. Todd suggested Gobind. Shrum approved, and in 1952, Esther and Gobind were married and moved to Vancouver.

Over the next eight years, Esther and Gobind had three children, and Esther provided a foundation and bearing for Gobind that sustained him for the next nearly 50 years until she died in 2001 from renal cancer. Esther's warmth and strength extended into the laboratory environment. Her death in many ways marked the end of Gobind's productive scientific career, since his own health deteriorated over the remainder of his lifetime after Esther's passing. The story of Esther and Gobind—how they met, how their early relationship survived, how they persevered despite the huge obstacles of geographical and cultural differences—and the challenges they must have faced together, especially in those early years, are a part of the personal story of Gobind's life that will fade into history largely untold.

Gobind's remarkable life and scientific achievements resulted, at least in part, from his extraordinary intellectual agility and personal integrity. He walked a tightrope between scientific cultures and disciplines. He had the intellectual 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 expanded or extended beyond the laboratory. He did not lead a department or start companies, but he continued to re-invent himself at regular intervals for more than 50 years, and in the process left a remarkable trail of technical achievements—some of which were truly transformative.

Although Gobind came under the influence of a roster of scientific luminaries during his early training, including Todd and Prelog, the real turning point in his career came in 1954 when he was working in Vancouver with just one or two students on the publication of the chemical synthesis of ADP and ATP using the carbodiimide reaction (Khorana 1954). In the 1940s, if you wanted a sample of pure ATP, you would begin your preparation with a live rabbit—seriously (Kerr 1941). Gobind later synthesized cyclic nucleotides, asymmetric dinucleotides, and other molecules of biological significance. Over time, with the help of several notable colleagues (Gordon M. Tener, John G. Moffat, Michael Smith, and others) at the British Columbia Research Council, every significant known nucleotide and nucleotide cofactor was synthesized. During summer "vacations" notable scientists, including Paul Berg, Arthur Kornberg, Eugene Kennedy, and many others, visited Gobind's laboratory to learn how to prepare and use the new carbodiimide reagents.

The culmination of the work in 1960 was the synthesis of Coenzyme A, by far the most complex of the nucleotide cofactors. In a remarkable concluding statement, Moffat and Khorana (1961) wrote:

> The synthesis of Coenzyme A and the work reported in the accompanying papers bring to a close the program of research initiated in this Laboratory some seven years ago on the development of methods for the synthesis of unsymmetrical pyrophosphates of biological interest. The methods available are believed to be satisfactory and completely general . . .

But Gobind had something else in mind. He moved to the Institute for Enzyme Research at the University of Wisconsin–Madison, his sights set even higher—chemical synthesis of a gene. His line of thinking was clearly outlined in 1960 with a provocative exhortation:

> 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? (Khorana 1960)

Gobind's decision to move forward with plans to synthesize a functional gene even when only dinucleotides could be made in satisfactory yield and before there was a reliable method to sequence DNA is almost unfathomable today in this era of short-term funding commitments. And as usual Gobind was not proposing to establish a major institute with corporate sponsors; he was simply committing to chip away, relentlessly, until he accomplished his goal. And once Gobind committed, there was no turning back—ever. He thought constantly about effort and commitment, sometimes doubling down when projects seemed hopeless. But he also seemed to know when to quit, or better stated, when to move on to other work, sometimes related, but often totally new.

In 1972 Gobind's team, now at the Massachusetts Institute of Technology, described the total chemical synthesis of a functional tRNA gene in an unprecedented and still unsurpassed achievement in

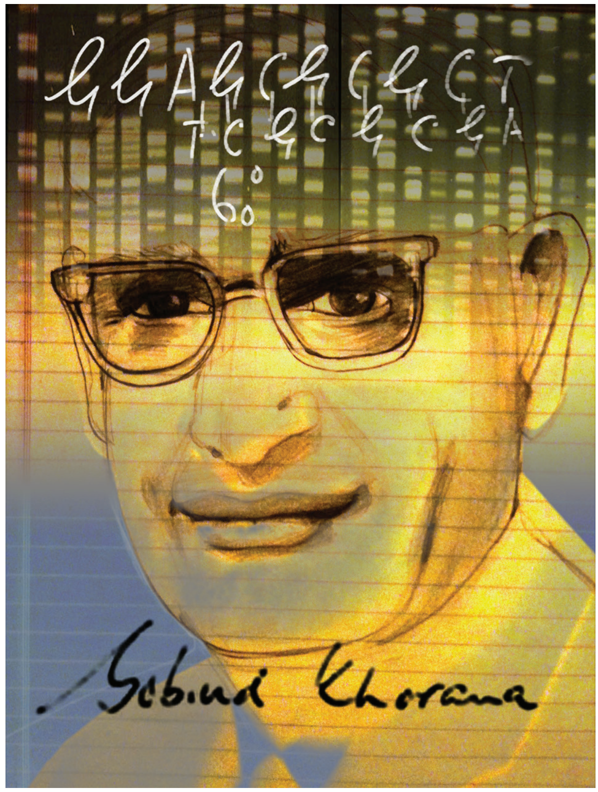

FIGURE 1. Prof. H. Gobind Khorana was one of the founders of the field of chemical biology, invented useful methods for DNA synthesis, was responsible for the first chemical synthesis of a functional gene, and contributed to the elucidation of the genetic code. Original illustration: Karina Åberg.

chemical biology, which was published in an entire issue of the *Journal of Molecular Biology* in December 1972—15 consecutive articles, 313 consecutive pages. The achievement was even more striking if we stop to consider that when the project was initiated, in 1960, there was no reliable method to synthesize more than a dinucleotide in reasonable yield nor, as mentioned earlier, was there a way to sequence DNA. The report of “nearest-neighbor” analysis by Kornberg—a test to confirm the sequence of the bases during replication—was all that Gobind needed to commit to the work.

And in the meantime, in one of the most significant scientific “detours” of all time, Gobind contributed fundamentally to the elucidation of the genetic code—one of the great scientific achievements of molecular biology (Figure 1). Energized by the Nirenberg and Matthaei experiment from 1961, where a cell-free extract produced a protein made entirely of phenylalanine when poly(U) was added, Gobind’s group in Madison worked around the clock in double shifts to synthesize all the possible triplet trinucleotides, thus providing a firm basis to establish the complete codon assignments and to determine how the code was read.

I remember vividly the first time I met Gobind, and I guess that anyone who has ever met Gobind would remember him. He was not necessarily what you would expect given his achievements. He seemed to have time; he listened and had no air of pretense at all. He preferred to be on the receiving end, to acquire more information to store, synthesize, and later recall using his prodigious memory. By the time I had met him at MIT in 1984, he was already 62 years old—a giant from an earlier time. But he gave no impression at that initial meeting that he was satisfied with his achievements or finished with his work. And in fact, he wasn't. He was active for 23 more years and published seminal work on transmembrane signaling and energy transduction.

With boldness and audacity that propelled him forward, Gobind would study and seek out the leaders of each successive field of interest and then, in most cases, leap beyond them while leaving a trail of highly accomplished colleagues (perhaps 250 postdoctoral fellows and students over the years), who would often go on to become leaders in whatever field he had jettisoned earlier.

The best example of this is when he switched from working primarily on nucleic acids and gene synthesis and took up the challenging subject of membrane proteins in the mid-1970s. After nearly a year traveling and visiting the leaders in the field, he chose to work on bacteriorhodopsin, the light-driven proton pump from patches known as the purple membrane. Within about five years, he would publish its complete amino acid sequence—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 Michael Smith) to elucidate mechanism. Related work on the G protein–coupled receptor (GPCR), rhodopsin, would follow. And many of the methods he reported, including the use of immunoaffinity purification and other approaches, were used later to advance the structural biology of GPCRs.

As a member of Gobind's group at MIT in the late 1980s, I saw a man still totally focused on laboratory work, following an almost relentless schedule, with 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 MIT, contributed immeasurably to Gobind's success and longevity after 1970.

Gobind exuded an almost childlike enthusiasm and energy, but with a laser-like focus. He would listen intently, tilting his head ever so slightly and leaning in toward you. Then something would click and he would leap up from his chair and bound toward a filing cabinet. Within seconds he would pull out a relevant set of notes, or a reprint. His files were almost exclusively organized by scientists' names—no need for key words or alphabetical subject lists. For Gobind, science was deeply personal, and his focus, intensity, and attention to detail characterized his approach to work, and propelled him from a small village in India, to post–World War II Europe, to Canada, and then to Madison and Cambridge—from those initial forays with the carbodiimide reaction to the genetic code to the synthesis of a gene.

The story of H. Gobind Khorana is the story of a great chemist and innovator who traveled one of the most remarkable personal and scientific journeys of the 20th century. With Gobind's death everyone who knew him and worked with him feels an immense sense of loss and must grieve the passing of a truly great man.

Elected 1973

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Author's Note

This tribute includes portions from earlier obituaries published in *ACS Chemical Biology* 17 (2012): 250–1 and *PLOS Biology* 10 (2012): e1001273.

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