BIOGRAPHICAL MEMOIRS

Har Gobind Khorana

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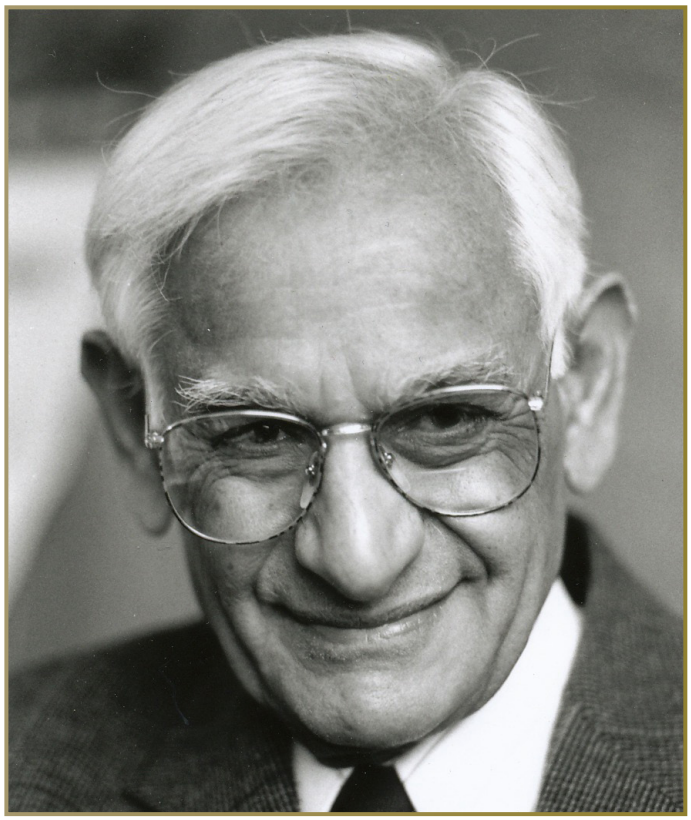
A Biographical Memoir by Phillip A. Sharp and Uttam L. RajBhandary



Har Gobind Khorana is a towering figure in the history of molecular biology and, arguably, one of the most notable chemists of the twentieth century. Pioneering contributions to the elucidation of the genetic code and synthesis of DNA and RNA with defined sequences are part of this legacy. He is the father of synthetic biology, first, for chemical synthesis of short DNA segments of specified sequences and using DNA polymerase to replicate these sequences and then transcribing this DNA template with RNA polymerase into RNA for use in protein synthesis[1] and, second, by joining short synthetic DNA segments into genes using DNA ligase.[2] This science laid the foundation for many groundbreaking discoveries and for the development of the biotechnology industry. Later, his pioneering work on proteins with seven transmembrane helices also paved the way for generations of membrane biologists to follow and ushered in what he called "the golden age of integral membrane proteins."

The first chemical synthesis of a gene, coding for a tRNA, was achieved in 1970 and a fully active tRNA gene with all the necessary sequences for its expression was completed in 1979.[3,4] This science, chemical in nature and driven by emerging concepts in molecular biology, was pivotal in extending chemistry into the domain of biology and created an essential part of the recombinant DNA revolution of the mid-1970s. These remarkable achievements overshadowed a life story of humble origin in a small village in India, training in the United Kingdom and Germany during times of great societal turmoil in his home country, and immigration to Canada and then the United States.[5]

Early Life and Education

Khorana was born on January 9, 1922, in Raipur, a small village in the Punjab province of India. His family was the only Hindu family in Raipur, where all the other inhabitants were Muslim. His father was a local tax collector, one of the few educated people in the village. Khorana and his siblings attended school in the village and then in a larger town nearby. He later won a scholarship to Punjab University in Lahore, where he obtained his baccalaureate degree in chemistry in 1943 and his master of science degree in 1945. As the result of the end of British colonial rule and the Partition

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of India and Pakistan in August 1947, Raipur became part of Pakistan. To escape the burgeoning sectarian violence, the Khorana family migrated from Pakistan to India essentially as refugees, with some members locating near New Delhi.

Khorana's mentees describe him as a "dedicated, driven, focused, and humble scientist." He was also a visionary in anticipating the importance of molecular biology and the role of chemistry in its advancement. Khorana's prescription for gaining this sense of foresight and intuition is found in the first few sentences of his autobiographical collected papers.[6] In describing his early inspirations, he quotes Nobel laureate Otto Warburg, who had been a student of legendary chemist and Nobel laureate Emil Fischer, "The most important event in the career of a young scientist is personal contact with the great scientists of his time." Indeed, Khorana's own scientific trajectory was greatly affected and influenced by the accomplished senior scientists who provided his initial scientific training and by other major figures of science with whom he associated later. The three scientists he worked with in his early years were all organic chemists: Roger J. S. Beer at the University of Liverpool, Vladimir Prelog at the Swiss Federal Institute of Technology (Eidgenössische Technische Hochschule, or ETH) in Zürich, Switzerland, and Alexander R. Todd at the University of Cambridge in the United Kingdom.

After obtaining his master's degree, Khorana was awarded a fellowship from the Indian government for graduate studies in England. The fellowship, sponsored by the Ministry of Agriculture, was intended for the study of insecticides and fungicides. But because of the large number of war veterans going back to college, there were limited opportunities for him at universities in England. Fortunately, a position became available in the Department of Chemistry in Liverpool for research in organic chemistry. At the time, the department was led by Alexander Robertson, a natural products chemist, who would be awarded the Davy Medal of the Royal Society in 1952. Khorana arrived in 1945 as World War II was ending and England was struggling to return to a new normal. With Roger Beer (who had earned his Ph.D. at Oxford University), as his supportive and attentive mentor in organic chemistry, Khorana completed his Ph.D. degree in 1948.[7]

Aside from being a scientific teacher and advisor, Beer treated Khorana almost as a member of his family, helping Khorana to get settled in a country so different from his small village in India. Of Roger Beer, Khorana said, "He supervised my research, and, in addition, looked after me diligently. He was my introduction to Western civilization and culture."

After finishing his Ph.D., and realizing that a substantial amount of the chemical literature at that time was in German, Khorana was determined to learn the language firsthand. He therefore decided to seek a postdoctoral position with Vladimir Prelog in Zürich. Prelog had moved to the ETH in 1941. Alkaloids were Prelog's interest at the time, and he had recently begun to separate and characterize stereoisomers of organic components—research for which he was recognized with the Nobel Prize in Chemistry in 1975. Khorana, with his Ph.D. in hand, approached Prelog despite having neither letters of recommendation nor any other introduction and asked for an opportunity to work in his laboratory. Fortunately, Prelog accepted him. Khorana could only stay a year because he supported himself during this period with savings from his time as a student in Liverpool. Khorana considered his year at the ETH to be a critical juncture in his career and said, "Vladimir Prelog made me see beauty in Chemistry, work and effort" and "I believe spending a year in Zürich was probably the wisest thing I ever did in my life."[8]

Khorana's strong determination to learn the German language also had another major impact on his future research. While reading papers in German during breaks in his lab work, Khorana came across a paper by Fritz Zetzsche on carbodiimides, reagents reactive with carboxylic acids.[9] One of these carbodiimides, hitherto unknown to most organic chemists of the day, played a central role in much of Khorana's subsequent research.[10,11] George W. Kenner had arrived in Prelog's laboratory from Cambridge at about the same time as Khorana, shared an adjacent bench, and worked on the same problem with Khorana—the structure of the erythrina alkaloids, the active compounds in arrow poison used by the indigenous peoples of the South American rainforest.[12] Kenner also had a critical role in the next step in Khorana's research career. Khorana left Zürich to find a position in India in 1949, but without success. Although it was two years after India had undergone Partition, creating Pakistan as a Muslim country, it was still a time of continuing and widespread civil unrest in India. Fortunately, through correspondence with Kenner, who had returned to Alexander Todd's laboratory in Cambridge, Khorana learned that Todd had funding for a position for research on peptides related to adrenocorticotropic hormone (ACTH). With Kenner's support, Khorana was offered a position with Todd, and he arrived in Cambridge in December 1949.

In Todd's group, Khorana was reunited with Kenner, and they worked on developing methods to synthesize and degrade peptides.[13] Peptide chemistry was rapidly advancing through techniques developed by eventual Nobel laureate Frederick Sanger and others. Recalling Zetzsche's carbodiimide paper, Khorana initiated research to degrade peptides from their carboxyl terminus. He successfully explored different types of carbodiimides, but not to the degree necessary to establish a general method.[14] But soon after, he made the breakthrough discovery that N,N'-dicyclohexylcarbodiimide (DCC) activated phosphate esters to produce

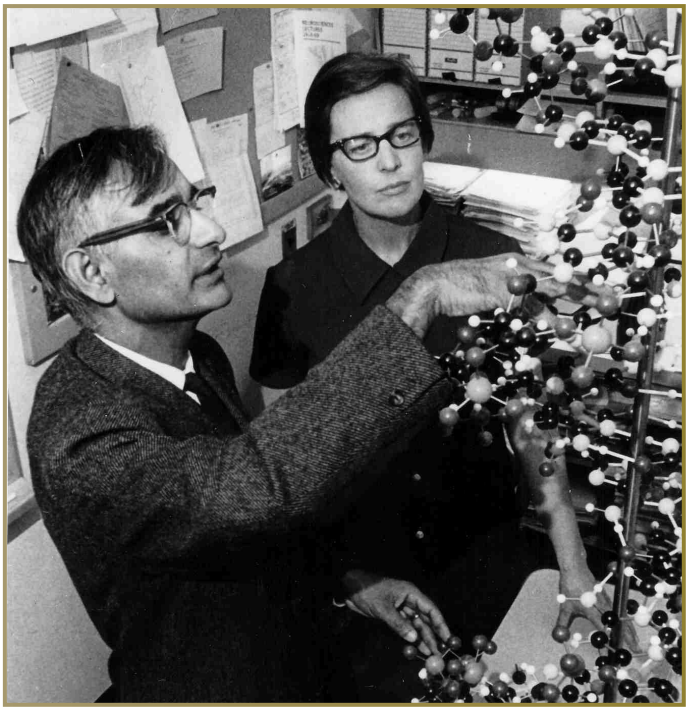

Figure 1 Gobind and his wife Esther looking at a skeletal molecular model.

pyrophosphates.[15] The finding that DCC could catalyze such a coupling reaction efficiently generated a great deal of excitement in Todd's laboratory, because chemical synthesis of pyrophosphates was a problem of central interest there. Todd's research on nucleotides and nucleotide coenzymes was recognized six years later by a Nobel Prize in Chemistry. Of his experience in the Todd laboratory, Khorana said, "It was exciting to be in Cambridge, the stay broadened my intellectual horizons and was of decisive value in my scientific development."

Move to Canada

Khorana left Cambridge in 1952 after being recruited to the Canadian province of British Columbia by Gordon M. Shrum, then head of the Research Council of British Columbia. In the same year, Khorana married Esther Elizabeth Sibler, a Swiss citizen whom he had met in 1947 during a visit to Prague.

Esther was a major force in his life, sharing with him her appreciation of European culture and art along with strongly encouraging his work. Khorana acknowledged the value of Esther's help in the preface to his book *Chemical Biology*; he states, "Esther gave me unfailing support throughout my scientific career. Taking care of all matters outside of the lab, she left me completely free to pursue my scientific work."[16] During their years in Canada, Esther gave birth to their three children—Julia Elizabeth, Emily Anne, and Dave Roy—and he established a nucleotide chemistry laboratory that attracted visits from the leading scientists in the new field of molecular biology.

While setting up his new laboratory in Canada, Khorana published in 1952 a masterly review, entitled "Structural Investigation of Peptide and Proteins," in *Quarterly Reviews*.[17] Thereafter, however, he focused his attention on the chemistry of carbodiimides, commencing with a review on this topic in *Chemical Reviews* in 1953.[18] By 1954, Khorana had extended his research on carbodiimides to a new pathway for synthesis of adenosine di- and triphosphates. This research attracted

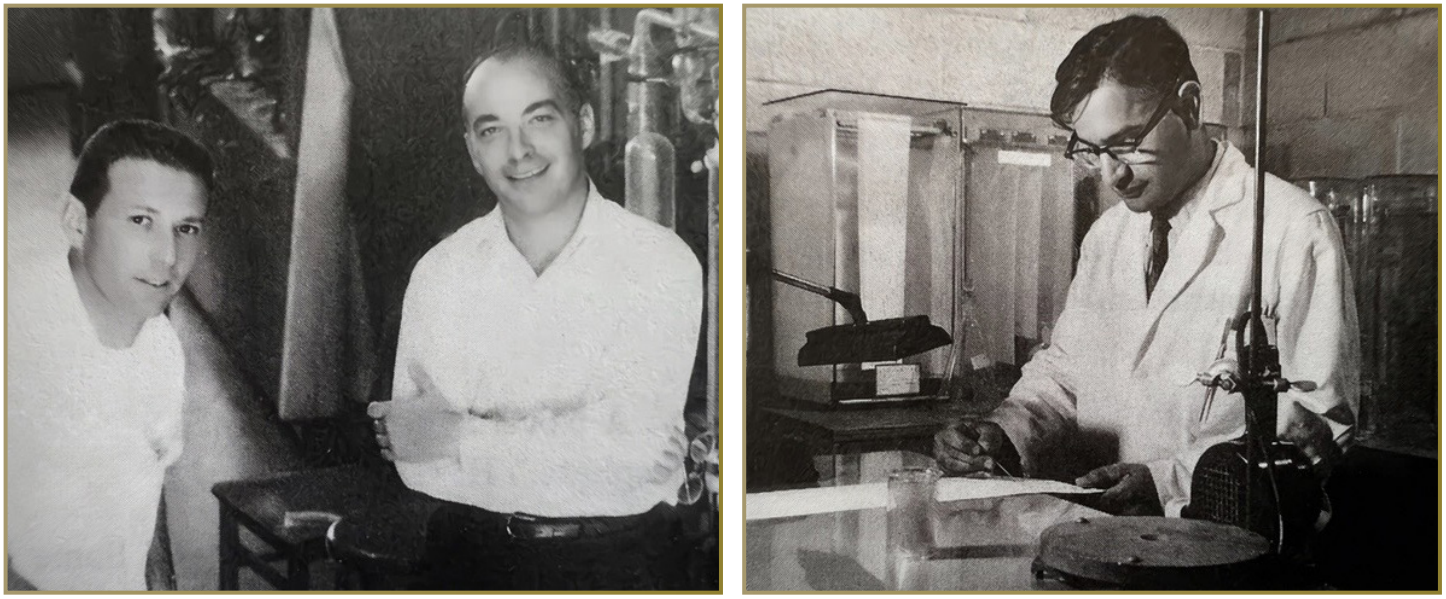

Figure 2 Left panel, Arthur Kornberg, right, and Paul Berg, left, on a working visit to the Khorana laboratory in Vancouver, B.C., during the summer of 1956. Right panel, Khorana working in his Vancouver laboratory, preparing to run a paper chromatogram to separate nucleotides.

leading nucleic acid biochemists, including eventual Nobel laureates Arthur Kornberg and Paul Berg and many others, to visit his laboratory to learn this technology. Through to 1958, Khorana continued with the use of carbodiimides for the synthesis of nucleoside triphosphates,[19] nucleotide coenzymes including Coenzyme A,[20] 3',5'-cyclic-AMP and 3',5'-cyclic GMP,[21] as well as important intermediates in metabolism, such as phosphoribosyl pyrophosphate.[22] Perhaps most significantly, these methods allowed Khorana to prepare both ribo- and deoxyribo-oligonucleotides of defined sequence, providing the tools that led to elucidation of the genetic code and the construction of synthetic genes.[23,24]

In 1958, Khorana attended the Gordon Research Conference, which had previously focused on both proteins and nucleic acids. Organized and chaired by Paul C. Zamecnik of Harvard Medical School, Khorana later recalled the event as epoch-making. It was the first Gordon Conference focused almost completely on nucleic acids, held five years after the announcement of the model for the structure of DNA by James D. Watson and Francis H. C. Crick. This five-year span was a period of intense research focused on understanding the nature of messenger RNA and on deciphering the genetic code. Like Khorana, other conference attendees were leaders in the field, including many Nobel laureates and future Nobel laureates, such as Fritz A. Lipmann, Crick and Watson, and Robert W. Holley, who along with Marshall W. Nirenberg would share the Nobel Prize in Physiology or Medicine with Khorana in 1968. Other luminaries included Paul Berg and Alexander Rich, Khorana's future colleague at the Massachusetts Institute of Technology (MIT). Forty years later during a session at MIT, in a display of his prodigious memory, Khorana stood before a blackboard and was able to sketch in detail the presentations given at that momentous 1958 meeting.

In 1960, Khorana was recruited to be one of three co-directors of the Institute for Enzyme Research at the University of Wisconsin, Madison, and became a naturalized U.S. citizen. Just one year later, at a meeting in Moscow, Marshall Nirenberg reported that polyribouridine ("poly-U") encoded polyphenylalanine in a cell-free lysate of the bacterium *Escherichia coli*.[25] Khorana maintained an ongoing association with Kornberg and Berg, with visits to their laboratories to learn DNA replication with DNA polymerase and purification of DNA-dependent RNA polymerase, which allowed Khorana to design a novel strategy to decrypt the genetic code—using synthetic RNAs with defined sequences to program cell extracts for protein synthesis. In Khorana's lab, short synthetic DNA oligonucleotides carrying known repeating di-, tri-, and tetra-nucleotide sequences were replicated by DNA polymerase and the resulting DNA polymers were transcribed with RNA polymerase to generate single-stranded

Figure 3 Khorana at an impromptu celebration of his 1968 Nobel Prize in Physiology or Medicine at the Institute for Enzyme Research at the University of Wisconsin, Madison, talking to biochemists and colleagues Henry A. Lardy, right, and Robert M. Bock, left.

RNAs. The use of such RNAs as mRNA in a cell-free system, followed by analysis of the polypeptides produced, enabled Khorana to determine the codon(s) that specify many of the twenty amino acids.[26,27] In parallel, Khorana synthesized all sixty-four ribo-trinucleotides and used them in a ribosome-binding assay[28] developed by Nirenberg and Philip Leder to establish codons for the remaining amino acids by identifying the specific aminoacyl-tRNA (then called "soluble" RNA or sRNA) that each trinucleotide attracted to the ribosome.[29,30] This work, along with that of Nirenberg, Nobel laureate Severo Ochoa, and earlier work by Zamecnik, Crick, and his associate and eventual Nobel laureate Sydney Brenner, and others, established that each codon of the genetic code consists of a triplet of nucleotides, assigned the amino acids encoded by sixty-one of the possible sixty-four codons, and demonstrated that the remaining three codons—dubbed amber (UAG), ochre (UAA), and opal (UGA)—terminate protein synthesis. For their unique contributions to elucidation of the genetic code, Khorana and Nirenberg were each recognized with a one-third share of the 1968 Nobel Prize in Physiology or Medicine along with Holley (who had determined the first nucleotide sequence for a tRNA).

There is an interesting story about how Khorana learned that he had been awarded the Nobel Prize. Among his colleagues in the lab and at the University of Wisconsin, he was the last to know. On the morning of the day the prize was announced, Khorana was away from home. As he often did while writing papers or thinking deeply about scientific problems, he had rented a small cottage by a lake located about an

hour away that had no radio or television. His wife, Esther, had to drive to the cottage, convey to him the good news, and bring him to the lab in the late morning. The impromptu celebration that followed later in the day highlights Khorana's intense focus on his latest research. The photo shows him talking to two of his faculty colleagues at the University of Wisconsin, not about his work on the genetic code that led to his share of the Nobel Prize, but rather his plans for his next goal, the chemical synthesis of a gene.[31]

Gene Synthesis

Total chemical synthesis as a critical step in confirming the structure of a natural product, and opening thereby avenues to investigate its activities, is a long-standing tradition in organic chemistry. In the 1960s, Khorana began thinking about gene synthesis at a time before the DNA corresponding to any gene had been isolated and characterized. In 1965, the only gene product of known sequence was yeast alanine tRNA[32]; consequently, DNA encoding this RNA became the target for Khorana's gene synthesis. The discovery of polynucleotide kinase in 1965 and DNA ligase in 1967 by several groups greatly facilitated the gene synthesis work by allowing the joining of oligonucleotides aligned next to one another as part of a DNA duplex. Khorana showed that short synthetic DNA oligonucleotides five to twenty units in length could be phosphorylated at the 5'-end and assembled to form longer, nicked, double-stranded DNA on the basis of sequence complementarity. DNA ligase could then be used to seal the nicks in the DNA to yield two longer complementary DNA strands. Khorana also showed that such DNA duplexes carrying complementary sticky ends as short as four or five

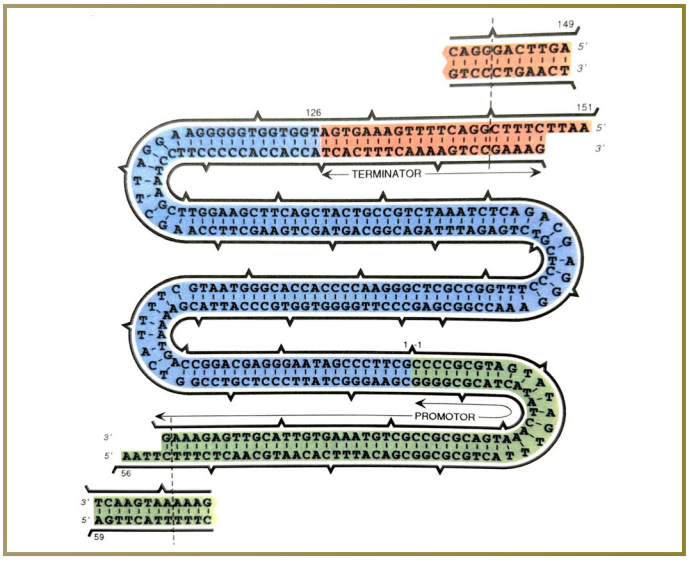

Figure 4 Synthetic gene for *E. coli* tyrosine amber suppressor tRNA. The structural gene (126 base pairs) is in blue, the promoter region (52 nucleotides) in green, and the terminator region (16 nucleotides) is in red. The single-stranded ends for ligase joining are shown.

nucleotides could be joined by DNA ligase to produce even longer DNAs. The synthesis of a 77-base pair DNA coding for the sequence of the alanine tRNA was announced in 1970.[33] This paper, considered to be a landmark in genetics, was followed by the publication of thirteen detailed articles in 1972 in an entire issue of the noted *Journal of Molecular Biology*. Khorana's work on gene synthesis provided a major part of the foundation of a field now dubbed synthetic biology.

Having synthesized the tRNA coding sequence, Khorana's next goal was to produce greater amounts of this synthetic DNA. Towards this goal, in a paper submitted in 1970 from Wisconsin, Khorana proposed a series of steps for DNA amplification that, in hindsight, seem remarkably similar to the now widely used polymerase chain reaction (PCR).[34] An excerpt from the last paragraph of this paper states:

> The principles for extensive synthesis of the duplexed tRNA genes which emerge from the current work are the following. The DNA duplex would be denatured to form single strands. The denaturation step would be carried out in the presence of a sufficiently large excess of the two appropriate primers. Upon cooling, one would hope to obtain two structures, each containing the full length of the template strand complexed with the primer. DNA polymerase will be added to complete the process of repair replication. Two molecules of the original duplex should result. The whole cycle could be repeated, there being added every time a fresh dose of the enzyme.…

It is surprising that this method of sequence amplification was not widely appreciated until eventual Nobel laureate Kary B. Mullis and his co-workers published their 1985 paper in which they described a virtually identical approach.[35]

At Wisconsin, Khorana also began work to synthesize a fully functional amber suppressor tRNA gene; but in 1970, he was recruited to MIT, where he completed synthesis of an active gene encoding E. coli tyrosine amber suppressor tRNA and spent the remaining years of his career.[36] This technology and science provided a critical part of the foundation for the recombinant DNA revolution of the mid 1970s and the launching of the first biotechnology company, Genentech, in 1976.

Synthesis of the first biologically active tRNA gene, yet another landmark in genetics, was reported by Khorana in 1979.[37] This paper was preceded by twelve consecutive papers in a single 1976 issue of the *Journal of Biological Chemistry* and followed by six consecutive papers in 1979 in the same journal. The project had begun in 1968 with selection of the *E. coli* tyrosine amber suppressor tRNA gene present in a variant of the bacteriophage phi 80, Φ80pSu3, which

carried the amber suppressor tRNA gene.[38] Khorana determined the promoter sequence necessary for transcription of the tRNA gene and added it to the synthetic tRNA gene, along with sequences at the 5′- and 3′-ends of the tRNA necessary for nucleolytic processing of the precursor tRNA to yield mature tRNA. The synthetic tRNA gene was 208-base pairs long and was shown to be active in suppressing amber stop codons. Other genes synthesized by Khorana encoded bacteriorhodopsin (1981) and its mutants[39]; rhodopsin, a G protein-coupled receptor, and its mutants[40]; and the α-subunit of transducin, a G protein.

Membranes and Membrane Proteins

At the pinnacle of his career and while still working towards synthesis of a functional suppressor tRNA gene, Khorana became interested in membranes and membrane proteins as critical objectives to advance research in molecular neurobiology and signal transduction. Here too, before initiating his work on membranes, Khorana spent a sabbatical in the laboratory of Efraim Racker, an expert in the field, to learn about detergents, micelles, vesicles, and proton pumping by bR. He visited Racker's lab many times and said, "Ephraim Racker introduced me to membrane biochemistry."

Eventually, Khorana decided to focus first on bacteriorhodopsin (bR), a light driven H^+ transporter coupled to ATP synthesis in *Halobacterium salinarum*, and then on rhodopsin, the light-sensing receptor of the vertebrate retina. These two proteins both comprise seven transmembrane helices and both use retinal as the chromophore. Rhodopsin is a prototypical representative of the family of G-protein coupled receptors (GPCRs), of which there are about 750 encoded in the human genome.

Integral membrane proteins are extremely difficult to work with. They are often highly hydrophobic and insoluble in aqueous solutions. Through sheer determination and careful systematic analysis, Khorana showed that bR could be completely denatured and then, amazingly, refolded to an active form in the presence of phospholipids, detergents, and its retinal chromophore.[41,42] This discovery came as a total surprise to many membrane biologists. Most importantly, it enabled the first systematic analysis of the effect of site-specific mutants of membrane proteins on their structure and function and thereby opened up the field of membrane biochemistry. Using this approach, Khorana identified the role of many of the amino acids in bR structure and function, notably the key role of two amino acids Asp85 and Asp96 on proton translocation across the membrane.[43,44]

Through his rhodopsin research, his discovery of dodecyl maltoside as an efficacious detergent for solubilization and immuno-affinity purification of rhodopsin paved the way for many others to apply this approach in their own work with other GPCRs and membrane proteins.[45] Another major finding from Khorana's work in this area was the identification of two cysteines in rhodopsin that form a disulfide bond critical for its tertiary structure.[46] These two cysteines, and the disulfide bond they form, are now known to be important for the tertiary structure of a great many other GPCRs, in particular those, like rhodopsin, in the Class A sub-family and those, like the receptor for glucagon and other peptide hormones, in the Class B sub-family. Khorana also identified amino acids in rhodopsin critical for light-dependent signal transduction through activation of its G-protein transducin, and the role of whole-body transmembrane helix movement in signal transduction.[47,48]

It is impossible to truly understand an individual's creative success, but how did Khorana accomplish so much? Although a very humble and a modest person, Khorana was also a believer in the motto of Nobel laureate Otto Loewi: "We must be modest except in our aims." So, to pursue the science he loved, Khorana took considerable personal risk, first leaving his home country and immigrating to the United Kingdom to pursue his advanced education, and later emigrating to Canada and then the United States to further his independent career. He had great confidence in his judgement about the role of chemistry in molecular biology, and he aimed for seemingly unsurmountable goals and never turned back, although his research focus changed several times in his career. In addition to having immense creative energy, perseverance and intellectual tenacity, he also prepared himself meticulously for the projects that he was undertaking by visiting the laboratories of leaders in the field to become personally familiar with current methodology. Most importantly, he and his colleagues strove for excellence. Generations of pre- and postdoctoral scientists trained by Khorana have made their own marks, abiding by Khorana's scientific credo, with its emphasis on creativity, dedication, hard work, focus, and stringency. Thus, Khorana's legacy lives on.

Outside of his academic interests, Khorana liked to hike, snowshoe, and listen to Western classical music. He began swimming in Vancouver, and once proficient, he wanted to excel. Pretty soon he was swimming in the ocean. At MIT, he was for many years a daily visitor to the MIT swimming pool, just across the courtyard from his office. For a time, he also spent a month or so every year visiting the laboratory of one of his former postdoctoral associates at the National Cancer Center Research Institute in Tokyo, Japan. He spent most of the time there writing papers, just thinking about the research underway in his lab or visiting with many of his former mentees. Khorana passed away on November 9, 2011, at the age of eighty-nine.

Acknowledgments

We thank Marvin Caruthers, Tom Sakmar, and Sadashiva Karnik for comments and suggestions on this biographical memoir and Julia Khorana for the original photographs of Figures 1 and 2.

Note

Given the limit on the number of citations allowed in NAS Biographical Memoirs, we are unable to include references to all of Khorana's relevant papers. We refer the reader to additional pertinent papers that can be found in references 1, 6, 31, and 41.

References

1 Khorana, H. G. 1968. Nucleic acid synthesis in the study of the genetic code. Nobel Prize Lecture, p. 196–220.

2 Agarwal, K. L., et al. 1970. Total synthesis of the gene for an alanine transfer ribonucleic acid from yeast. *Nature* 227:27–34.

3 Agarwal, K. L., et al. 1970.

4 Khorana, H. G. 1979. Total synthesis of a gene. *Science* 203:614–625.

5 Khorana, H. G. 2000. A life in science. *Science* 287:810.

6 Khorana, H. G. 2000. *Chemical Biology: Selected Papers of H. Gobind Khorana (With Introductions)*. World Scientific Series in 20th Century Biology, Vol. 5. Singapore: World Scientific.

7 Beer, R. J. S., et al. 1948. The chemistry of the melanins. Part I. The synthesis of 5:6 dihydroxy-indoles and related compounds. *J. Chem. Soc.* 452:2223–2226.

8 Khorana, H. G. 1996. The year 1948-1949 in Prelog laboratory at ETH, Zürich: A reminiscence. *Croat. Chem. Acta* 69:417–421.

9 Zetzsche F., E. Lüscher, and H. E. Meyer. 1938. Labelling of carboxylic acid as ureid with the help of carbodiimides (I announcement). *Ber. Dtsch. Chem. Ges.* 71:1088–1093.

10 Khorana, H. G. 1968.

11 Khorana, H. G. 1981. From carbodiimide to gene synthesis. XIIIth Annual Feodor Lynen Lecture, Miami Winter Symposium.

12 Prelog, V., et al. 1949. Erythrina-alkaloide. 1. Mitteilung. Über erythralin und erysodin, die hauptalkaloide der erythrina abyssinica lam. *Helv. Chim. Acta* 32:453–461.

13 Kenner, G. W., and H. G. Khorana. 1952. Peptides. Part II. Selective degradation by removal of the terminal amino acid bearing a free amino-group. The use of alkyl alkoxydithioformates (dialkyl xanthates). *J. Chem. Soc.* 386:2076–2081.

14 Khorana, H. G. 1952. Peptides. Part III. Selective degradation from the carboxyl end. The use of carbodiimides. *J. Chem. Soc.* 387:2081–2088.

15 Khorana, H. G., and A. R. Todd. 1953. Studies on phosphorylation. Part XI. The reaction between carbodi-imides and acid esters of phosphoric acid. A new method for the preparation of pyrophosphates. *J. Chem. Soc.* 65:2257–2260.

16 Khorana, H. G. 2000. *Chemical Biology: Selected Papers of H. Gobind Khorana (With Introductions).*

17 Khorana, H. G. 1952. Structural investigation of peptides and proteins. *Q. Rev.* 4:340–357.

18 Khorana, H. G. 1953. The chemistry of carbodiimides. *Chem. Rev.* 53:145–166.

19 Khorana, H. G. 1954. Carbodiimides. Part V. A novel synthesis of adenosine di- and triphosphates and P^1,P^2-diadenosine-5-pyrophosphate. *J. Am. Chem. Soc.* 76:3517–3522.

20 Moffatt, J. G., and H. G. Khorana. 1961. Nucleoside polyphosphates. XII. The total synthesis of coenzyme A. *J. Am. Chem. Soc.* 83:663–675.

21 Smith, M., G. I. Drummond, and H. G. Khorana. 1961. Cyclic phosphates. IV. Ribonucleoside-3′,5′ cyclic phosphates. A general method of synthesis and some properties. *J. Am. Chem. Soc.* 83:698–706.

22 Tener, G. M., and H. G. Khorana. 1958. Phosphorylated sugars. VI. Syntheses of α-D-ribofuranose 1,5-diphosphate and α-D-ribofuranose 1-pyrophosphate 5-phosphate. *J. Am. Chem. Soc.* 80:1999–2004.

23 Gilham, P. T., and H. G. Khorana. 1959. Studies on Polynucleotides. V. Stepwise Synthesis of Oligonucleotides. Synthesis of thymidyl-(5′→3′)-thymidylyl-(5′→3′)-thymidine and deoxycytidylyl-(5′→3′)-deoxy adenylyl-(5′→3′)-thymidine. *J. Am. Chem. Soc.* 81:4647–4650.

24 Smith, M., et al. 1962. Studies on Polynucleotides. XIV. Specific synthesis of the $C_{3'}$-$C_{5'}$ interribonucleotide linkage. Syntheses of uridyl-(3″→5″)-uridine and uridylyl-(3″→5″)-adenosine. *J. Am. Chem. Soc.* 84:430–440.

25 Nirenberg, M. W., and J. H. Matthaei. 1961. The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polyribonucleotides. *Proc. Natl. Acad. Sci. U.S.A.* 47:1588–1602.

26 Wells, R. D., E. Ohtsuka, and H. G. Khorana. 1965. Studies on polynucleotides. L. Synthetic deoxyribopolynucleotides as templates for the DNA polymerase of *Escherichia coli*: A new double-stranded DNA-like polymer containing repeating dinucleotide sequences. *J. Mol. Biol.* 14:221–237.

27 Nishimura, S., D. S. Jones, and H. G. Khorana. 1965. Studies on polynucleotides. XLVIII. The *in vitro* synthesis of a co-polypeptide containing two amino acids in alternating sequence dependent upon a DNA-like polymer containing two nucleotides in alternating sequence. *J. Mol. Biol.* 13:302–324.

28 Nirenberg, M., and P. Leder. 1964. RNA codewords and protein synthesis. The effect of trinucleotides upon the binding of sRNA to ribosomes. *Science* 145:1399–1407.

29 Lohrmann, R., et al. 1966. Studies on polynucleotides. LI. Syntheses of the 64 possible ribotrinucleotides derived from the four major ribo-mono-nucleotides. *J. Am. Chem. Soc.* 88:819–829.

30 Söll, D., et al. 1965. Studies on polynucleotides, XLIX. Stimulation of the binding of aminoacyl-sRNA's to ribosomes by ribotrinucleotides and a survey of codon assignments for 20 amino acids. *Proc. Natl. Acad. Sci. U.S.A.* 54:1378–1385.

31 Söll, D., and U. L. RajBhandary. 2022. Khorana, Har Gobind. In: *Reference Module in Life Sciences*, ed. W. C. Summers. Amsterdam: Elsevier, n.p., ol.

32 Holley, R. W., et al. 1965. Structure of a ribonucleic acid. *Science* 147:1462–1465.

33 Agarwal, K. L., et al. 1970.

34 Kleppe, K., et al. 1971. Studies on polynucleotides. XCVI. Repair replications of short synthetic DNA's as catalyzed by DNA polymerases. *J. Mol. Biol.* 56:341–361.

35 Saiki, R. K., et al. 1985. Systematic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230:1350–1354.

36 Khorana, H. G. 1979.

37 Khorana, H. G. 1979.

38 Russell, R. L., et al. 1970. Duplicate genes for tyrosine transfer RNA in *Escherichia coli. J. Mol. Biol.* 47:1–8.

39 Nassal, M., et al. 1987. Structure-function studies on bacteriorhodopsin. III. Total synthesis of a gene for bacterio-opsin and its expression in *Escherichia coli. J. Biol. Chem.* 262:9264–9270.

40 Ferreti, L., et al. 1986. Total synthesis of a gene for bovine rhodopsin. *Proc. Natl. Acad. Sci. U.S.A.* 83:599–603.

41 Fahmy, K., and T. P. Sakmar. 2022. Interdisciplinary biophysical studies of membrane proteins bacteriorhodopsin and rhodopsin. *Biophys. Rev.* 15:111–125.

42 Huang, K. S., et al. 1981. Refolding of an integral membrane protein. Denaturation, renaturation, and reconstitution of intact bacteriorhodopsin and two proteolytic fragments. *J. Biol. Chem.* 256:3802–3809.

43 Khorana, H. G. 1988. Bacteriorhodopsin, a membrane protein that uses light to translocate protons. *J. Biol. Chem.* 263:7439–7442.

44 Greenhalgh, D. A., et al. 1992. Effect of introducing different carboxylate-containing side chains at position 85 on chromophore formation and proton transport in bacteriorhodopsin. *J. Biol. Chem.* 267:734–25738.

45 Oprian, D. D., et al. 1987. Expression of a synthetic bovine rhodopsin gene in monkey kidney cells. *Proc. Natl. Acad. Sci. U.S.A.* 84:8874–8878.

46 Karnik, S. S., et al. 1988. Cysteine residues 110 and 187 are essential for the formation of correct structure in bovine rhodopsin. *Proc. Natl. Acad. Sci. U.S.A.* 85:8459–8463.

47 Khorana, H. G. 2000. Molecular biology of light transduction by the mammalian photoreceptor, rhodopsin. *J. Biomol. Struct. Dyn.* 17(Suppl. 1):1–16.

48 Farrens, D. L., et al. 1996. Requirement of rigid-body movement of transmembrane helices for light activation of rhodopsin. *Science* 274:768–770.