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Hargobind Khorana - the Legendary Biochemist who cracked the Genetic code

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ABSTRACT

Har Gobind Khorana, the Nobel Prize-winning scientist, would have turned 100 on 9th January, 2022. Many generations of chemists and biologists have been inspired by Gobind's works and intellectual elegance, but high visibility of his success has had a much broader and deeper impact on India. Gobind Khorana is a popular scientific personality in India who represents how education may assist people to transcend socioeconomic and intellectual barriers. His triumph was fought for, and implausible.

KEYWORDS: Har Gobind Khorana, Nobel Prize, Genetic Code, Protein Synthesis, Dicyclohexylcarbodiimide, Bacteriorhodopsin

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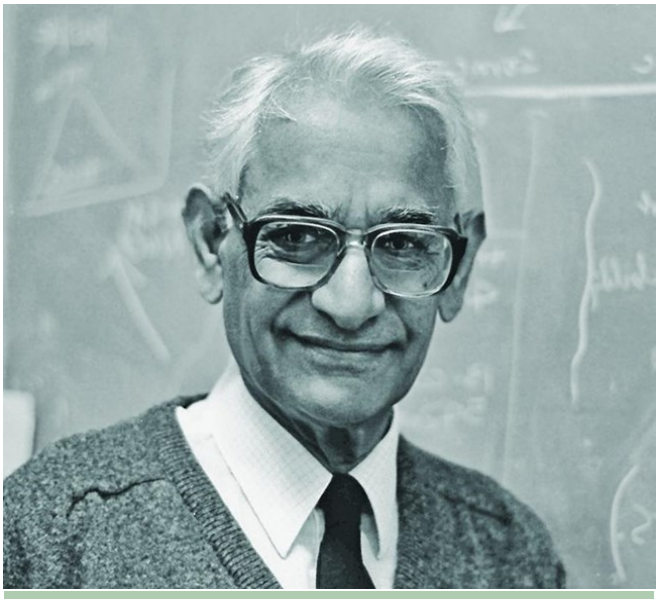
HUMBLE BEGINNINGS

Beginning with a low note, Raipur is where Har Gobind Khorana was born, a small village in Punjab, currently a part of eastern Pakistan, to Hindu parents. His exact date of birth is unknown; papers state that he was born on January 9th, 1922. He comes from a household with one girl and four sons. In the British Indian system of administration, his father was a "patwari," a village agricultural taxation clerk. Despite his family's poverty, Khorana's father insisted on his children receiving an education. He instilled in them the ability to read and write from an early age. The young Khorana went to school under a tree for his first four years until his father helped create a one-room school in their community. Har Gobind Khorana went to D.A.V. High School in Multan (today West Punjab); one of his professors, Ratan Lal, had a big influence on him during that time. Fortunately, his abilities were noticed early on, and he attended Punjab University in Lahore, where he almost majored in English since he was too nervous to interview for the Chemistry department. Nonetheless, due to his talent, the selection committee overlooked this, and he received a Master's degree in Chemistry. In 1945, he was awarded a rare fellowship to study Organic Chemistry at University of Liverpool in England.

EDUCATION AND FELLOWSHIP

Gobind travelled to the Eidgenossische Technische Hochschule (ETH) in Zurich after receiving his degree in 1948 to join the group of Vladimir Prelog, a Nobel Prize-winning chemist. During his brief time at the ETH, Gobind happened to read Fritz Zetzsche's work on carbodiimides, which he had never heard of before. This family of chemicals was critical for Gobind's ability to synthesize nucleotide cofactors like coenzyme A and ATP with "astonishing quickness," as he subsequently stated in his comments. After graduating from ETH, Gobind was unable to locate work in India, which favoured the continuation of his career in the west. He was awarded a three-year scholarship at Cambridge University under Lord Alexander R. Todd and G. W. Kenner. Khorana became interested in nucleic acids and proteins while at Cambridge. Sanger's dramatic advances in protein sequencing, Perutz and Kendrew's triumphs in protein crystallography, and Todd's own work on the chemical structures of nucleic acids were all exposed to Gobind while at Cambridge. Gobind, a synthetic organic chemist, was drawn to the nascent science of Molecular Biology by this inventive environment.



Har Gobind Khorana 1922-2011
Courtesy: C & EN - American Chemical Society

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SCIENTIFIC CAREER

Gobind began his independent scientific career in 1952 with the Research Council of British Columbia in Vancouver, Canada, as a non-academic researcher. Khorana was offered a job at a University of British Columbia in 1952, where he was permitted to research on esters of phosphate with nucleic acids, on his own. During this period, studies on the chemical synthesis of ribonucleotides for protein synthesis began. Gobind was co-Director of the "Enzyme Institute" and a member of the Department of Biochemistry at the University of Wisconsin-Madison, from 1960 to 1970. He kept working on synthesising nucleotides and unlocking the genetic code. The Nobel Prize for Physiology or Medicine was shared in 1968 amongst Hargobind Khorana, Robert Holley and Marshall Nirenberg for this study. From 1970 until his retirement in 2007, he worked as Alfred P. Sloan Professor of Biology and Chemistry at the Massachusetts Institute of Technology.

CONTRIBUTIONS WHICH MADE A BREAK-THROUGH IN SCIENCE

Scientist Gobind Khorana was a person who crossed both scientific and cultural boundaries. He was one of the first to apply the principles and tools of Chemistry and Physics to biological problems. He was especially instrumental in deciphering how RNA encodes for protein synthesis, for which he shared the Nobel Prize in Medicine with Marshall Nirenberg and Robert Holley in 1968. "I exclusively work on large problems," Gobind would often say, despite his modesty and quiet demeanour. Only such difficulties were worthy of Gobind's great energy, ingenuity, and attention when it came to a problem. He detailed the amplification of synthetic genes in a series of papers immediately after his departure from the University of Wisconsin-Madison to the Massachusetts Institute of Technology in 1971. With the invention of a thermostable DNA polymerase, 15 years later these steps were renamed PCR.

He was a pioneer in the area of Chemical Biology, as it is today called. He developed new synthetic pathways for nucleotide cofactors and oligonucleotides, which he subsequently used to help decipher the genetic code. The first synthetic gene was put together by him, effectively creating the first "biobrick" and laying the groundwork for Synthetic Genome and Synthetic Biology. During his tenure at University of Wisconsin-Madison, he synthesised oligonucleotides and bio- synthetically amplified these molecules with DNA polymerase. He discovered that the triplets CUC and UCU encode the amino acids leucine and serine respectively, using the oligonucleotide CUCUCU. Marshall Nirenberg had earlier proved that a UUU triplet encoded a phenylalanine residue, and this research backed up his findings. Gobind would always say, with his customary modesty, that Nirenberg's study inspired his own research into the genetic code. Gobind had already set his eyes on synthesising a whole gene by this time, despite the fact that his grasp of a gene was still sketchy. Gobind's team constructed the coding sequence of the alanine tRNA gene using DNA ligases. By 1976, Gobind had added the necessary regulatory components to allow the gene to be expressed in a bacterial cell that is alive, and he had proved that the synthesised tRNA functioned exactly like the naturally expressed gene. This masterwork established the conceptual and technical underpinning for biotechnology, and it is still the method used to construct synthetic genes and genomes four decades later.

At MIT, Gobind's second part of his career began. He concentrated on two main goals: understanding how intrinsic membrane proteins work and mapping their interactions with phospholipids in the lipid bilayer structure on a molecular level. Gobind became fascinated by light-sensitive microorganisms, with a particular fascination for Halobacterium halobium purple membranes. The characterization of H. halobium bacteriorhodopsin and, subsequently, mammalian rhodopsin, the last significant difficulty faced by the Khorana lab, was the result of this study. Gobind uncovered the detailed mechanism by which bacteriorhodopsin pumps protons through the membrane when stimulated by light with the help of innumerable peers and partners. The mechanism and structure-function relationships in mammalian rhodopsin were then determined using similar methods, a topic that had previously been unsolved.

UNRAVELLING THE GENETIC CODE

Khorana's research in Vancouver centred on the utilisation of dicyclohexylcarbodiimide (DCC) to create complex compounds, particularly enzymes, which are protein molecules that regulate metabolism. He was successful in generating ATP, the energy-producing molecule in cells. By 1960, he had created Coenzyme A, an even more complicated molecule. As a result of his achievements, he became one of the most influential biological chemists of his day. Khorana offered a stunningly ambitious goal - the fabrication of a synthetic gene – due to the fact that DCC also let a researcher to connect together DNA sequences. Nothing like it had ever been done before, and it became Holy Grail of Khorana's laboratory. In 1960, Khorana enrolled at the University of Wisconsin - Madison. Matthew Matthaei and Marshall Nirenberg, two biochemists, announced a means to interpret DNA in 1961.

Outside of a living cell, there is a technique by which DNA is transcribed into amino acids sequences. They started by putting several components of a cell in a test tube. Short DNA sequences were then put into the test tube, and the system translated them into a sequence of amino acids, which became part of a protein. Khorana was electrified by the effect. He was able to construct whatever DNA sequence he desired using the chemical DCC, which was beyond Nirenberg and Matthaei's capabilities. All of the DNA-encoded amino acid sequences could be produced by putting manufactured sequences inside a test tube. Several laboratories were vying for the same task. Khorana's team worked double shifts around the clock to crack the code first. It was finished in 1966.

AWARDS

Robert W. Holley, Har Gobind Khorana, and Marshall W. Nirenberg shared the Nobel Prize in Physiology or Medicine in 1968 for "their understanding of the genetic code and its function in protein synthesis." Khorana received the 1968 National Medal of Science, the Albert Lasker Basic Medical Research Award, and the Nobel Prize (1987).

KHORANA SCHOLARS PROGRAM FOR MOULDING YOUNG SCIENTISTS

The Khorana Initiative was established in his honour in 2007 by the UW- Madison, the Government of India, together with the Indo-US Science and Technology Forum, with the goal of bringing together scientists, businessmen, and social entrepreneurs from the US and India. Khorana Scholars is a programme that aims to mould young scientists. The year 2007 marked

Gobind's departure from active research, and University of Wisconsin - Madison established the Khorana Scholars Program, which was inspired by Gobind's tale. The initiative, which aspires to find and nurture future leaders in United States and India, was generously named after Gobind. This initiative seeks brilliant Indian and American students, as well as places them in top laboratories in the host country for a transformative summer research experience. The Khorana initiative is currently growing to include collaborations with top American colleges and renowned Indian research institutes *(http://www.biochem.wisc.edu/faculty/ansari/khorana program)*. The programme also deputes Wisconsin agricultural experts to India to assist in entrepreneurial efforts targeted at increasing economic stability and food security in disadvantaged rural communities. In 2009, Gobind met the scholars at his most recent visit to University of Wisconsin - Madison. He was ecstatic to meet the next generation of scientists because he could see their enthusiasm for research and possibly recognise a part of himself in them. Gobind motivated many of his pupils and they became leaders in industry and academia. In 1993, his postdoctoral fellow Michael Smith shared the Nobel Prize in Physiology or Medicine for site-directed mutagenesis with Kary Mullis, who was rewarded for inventing PCR. Other trainees went on to start large biotech businesses like Amgen and become presidents of prestigious universities and research centres.

A GREAT HUMAN BEING AND A MENTOR FOR MANY

Gobind's mentorship included rigorous intellectual training and hard effort. He was a great human being and a mentor for many. Even if none of the trials worked (at least for the unsolvable challenges!), complete "24/7" involvement was predicted. Gobind would bring doughnuts to the lab on Saturday mornings, according to one probably fictitious anecdote. It was rumoured that he had figured out that everyone's favourite type of food was a doughnut. He would only bring one doughnut each. He would check the remaining doughnuts at the end of the day to see who had come in over the weekend! The lab worked hard, but it also had many memorable Friday night gatherings in a room above the Muddy Charles pub at MIT, where spouses, children, and dogs were all welcome. Gobind's lab attracted researchers across disciplines and countries, engaging academic posts all over the world. He treated women and men equally and one of his former postdoctoral fellows went on to become one of Japan's first women Biochemistry professors. The respect and warmth that Gobind's academic family felt were evident in the "Khorana Symposia," which was held on a regular basis in a variety of locations across the world. The 33rd Steenbock Symposium on Synthetic Gene to Synthetic Genomes held in 2009 was the most recent such gathering. Majority of the scientists who spoke remarked about how Gobind influenced their perspective and his efforts are still propelling new fields like synthetic and chemical biology.

Har Gobind Khorana receives the Nobel Prize from King Gustaf Adolf in Stockholm

PERSONAL LIFE

Gobind was enamoured with nature and its beauty, and he actively sought isolation in beautiful locations in order to think profoundly and critically about science. Esther Elizabeth Sibler, a Swiss-born woman, and Har Gobind Khorana were wed in 1952. Khorana and Esther married and relocated to Vancouver. Khorana felt out of place everywhere and did not feel at home anywhere after six years away from his birth country. Esther brought a continuous sense of direction in his life. Dave Roy (born July 26th, 1958), Emily Anne (born October 18th, 1954), and Julia Elizabeth (born May 4th, 1953) are their three children. He could only focus on his profession because of his loving wife Esther, whom he lost in 2001. He would frequently rent a room or cottage without a phone, radio, or television so that he could concentrate on his writing and thinking. Esther actually had to travel one hour to tell Gobind of his Nobel Prize win. Gobind's daughter Emily died tragically in 1978. Julia and David looked after him in his later years. Har Gobind Khorana was a well-respected scientist, educator, and humanitarian. He devoted himself to his task with zeal, but he remained humble throughout. Those who were lucky enough to be his friends will remember his natural interest and appreciation for life, along with his rapid grin, slight tilt as he craned his head to listen, and curious expression. His memory will be passed down through the generations. The greatest place to search for an appreciation of Gobind Khorana's enormous scientific opus is a volume of selected papers with introductions published by Gobind a few years ago *(Khorana HG, 2000).*

LATER YEARS

Khorana's dogged quest of artificial genes took a detour when he cracked the genetic code. Khorana relocated his lab to the MIT in 1970, where he spent remainder of his life. He completed the whole synthesis of a functioning gene outside of a living organism in 1972. He also demonstrated that it may work in bacteria. Despite his acknowledged brilliance, Khorana's life was blighted by racism for the majority of his professional life. His daughter quoted that white citizens in the United Kingdom and Canada mocked him as a "Paki." He was paid less and expected to work more in Canada than his white counterparts. She also mentioned that their mixed-race family when in in the South of United States, wouldn't dare to travel together. H. Gobind Khorana passed away in Concorde, Massachusetts, on November 9, 2011.

BIBLIOGRAPHY

Ansari AZ, Rosner MR, Adler J (2011). *Har Gobind Khorana* 1922-2011. Cell.147(7): 1433-5.

Britannica, The Editors of Encyclopaedia. *"Har Gobind Khorana"*. Encyclopedia Britannica, November 5 2022, https://www.britannica.com/biography/Har-Gobind-Khorana. Accessed 10 December 2022.

H. Gobind Khorana – Nobel Lecture. Nobel Prize.org., https://www.nobelprize.org/prizes/medicine/1968/khorana/lecture. Accessed on Sat. 10 Dec 2022.

Har Gobind Khorana (1922-2011). DNA from the beginning. http://www.dnaftb.org/22/bio-2.html. Accessed 10 December 2022.

Khorana H. G. (2000). *Chemical biology : selected papers of h. gobind khorana (with introductions).* World Scientific. Retrieved December 21 2022 from http://site.ebrary.com/id/10699216.

Sahotra Sarkar (2022). *"Har Gobind Khorana: The chemist who cracked DNA's code and made the first artificial gene was born into poverty 100 years ago in an Indian village".* The Conversation, April 5, 2022, https://theconversation.com/har-gobind-khorana-the-chemist-who-cracked-dnas-code-and-made-the-first-artificial-gene-was-born-into-poverty-100-years-ago-in-an-indian-village-178390. Accessed on Sat. 10 Dec 2022.