Har Gobind Khorana

Early Years in Science and Transition from Chemistry to Chemical Biology

Uttam L RajBhandary

This article provides a brief summary of Har Gobind Khorana's early years in science including his years as an independent investigator at the British Columbia Research Council in Vancouver, Canada. It also includes interesting anecdotes about his career and about him as a scientist based on his own writings, including (1) how he simply showed up in the laboratory of Dr. Vladimir Prelog in Zurich, with no recommendations, and pleaded for a little space to do postdoctoral research under him, (2) how his determination and tenacity to learn the German language well introduced him to the world of a class of chemical reagents called 'carbodiimides', which proved pivotal for much of his early work on the synthesis of nucleotides, nucleotide coenzymes and the first synthesis of a gene, and (3) how he ended up in Vancouver, Canada for his first position as an independent investigator.

Har Gobind Khorana, whose scientific career spanned a period of more than sixty years, was a pioneer and a visionary. During this period, he published more than five hundred papers in chemistry and in biology. His early training was in chemistry but he soon found that his interest lay in applying chemistry to solve problems in biology. In doing so, he started the field of chemical biology. The switch from chemistry to chemical biology started while he was at the British Columbia Research Council (BCRC), University of British Columbia in Vancouver, Canada.

Khorana started his career as an independent investigator at BCRC in 1952 and soon began publishing prolifically on the synthesis of sugar phosphates, nucleotides and nucleotide coenzymes and started his pioneering work on the synthesis of DNA and RNA oligonucleotides. In 1960, Khorana moved from

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Carbodiimides, ATP, cAMP, cGMP, nucleotide coenzymes, Co-A, gene synthesis, genetic code.

Vancouver to the Institute for Enzyme Research at the University of Wisconsin in Madison. His major accomplishments at the University of Wisconsin were the elucidation of the genetic code, for which he shared the Nobel Prize in Physiology or Medicine in 1968 with Marshall Nirenberg and Robert Holley, and the first chemical synthesis of a gene, that for a transfer RNA (tRNA). In 1970, Khorana moved to the Massachusetts Institute of Technology (MIT), where he synthesized a suppressor tRNA gene and showed that the synthetic gene was functional in a bacterium, another landmark achievement in genetics. He then switched to working on membranes, membrane proteins and signal transduction, problems in which he worked for more than thirty years until his retirement in 2007.

In this article, I shall describe briefly the early years of Khorana's career as a scientist including his work at BCRC in Canada. My colleagues, Dieter Söll, Marvin Caruthers, Sriram Subramaniam, Sadashiva Karnik, David Farrens and Thomas Sakmar, are writing about his years at Wisconsin and at MIT.

Khorana's Early Years

Khorana was born in Raipur, a small village in the part of Punjab, which is now Pakistan. He received his bachelor's and master's degrees in organic chemistry from Punjab University in Lahore. While studying for his master's degree, he published two short papers as part of the *Lahore Philosophical Society* publications on the synthesis of xanthones with his supervisor Gurbaksh Singh [1]. In 1945, Khorana went to the University of Liverpool in UK on a Government of India Fellowship to do research for a PhD in organic chemistry.

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There is an interesting story on how Khorana ended up working for a PhD in organic chemistry. Because his fellowship came from the Ministry of Agriculture of the Government of India, Khorana was initially slated to work at an Institute in Berkshire, England, to study insecticides and fungicides. However, with the end of the Second World War, most educational institutions in

UK were crowded because of the influx of a large number of veterans returning home to finish their education. Since Khorana had a master's degree in organic chemistry, the Indian High Commissioner's Office in London decided that he might as well work for a PhD in organic chemistry instead of studying insecticides and fungicides. In Liverpool, Khorana worked on the chemistry of melanins and indoles under Roger Beer, published several papers [2], and received a PhD in 1948.

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For the next phase of his career, Khorana wanted to do postdoctoral research in a German-speaking country. Much of the chemical literature that he had come across in his PhD work was in German and he was determined to be proficient in the language. So he went to the Swiss Federal Institute of Technology in Zurich to seek a position as a postdoctoral researcher. According to Khorana, with his PhD thesis in his hand, he arrived at the office of Vladimir Prelog (a future Nobel Prize winner in Chemistry, in 1975) and pleaded for a little space to do research under him. Fortunately, Prelog accepted him and Khorana had a wonderful year in Zurich working on the chemical structure of Erythrina alkaloids, the active ingredients in some of the arrow poisons. He published two papers [3] from this work, both in German in the journal *Helvetica Chimica Acta*. Looking back at his time in Zurich, Khorana said "Vladimir Prelog made me see beauty in chemistry, work and effort." His stay at Zurich lasted only eleven months because Khorana received no stipend for his postdoctoral research and had to sustain himself on savings from his days as a PhD student at Liverpool University. About this Khorana said, "During the year in Zurich, with no subsidy, of course I had to be very careful with my living expenses; but looking back, I believe that spending the year in Zurich was probably the wisest thing I ever did in my life."

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Khorana's stay at Zurich had another monumental effect that was to influence his research for years to come. A hallmark of Khorana's character was to excel in everything that he did. In addition to his laboratory work on alkaloids, in his efforts to learn German well enough to speak it fluently and understand fully

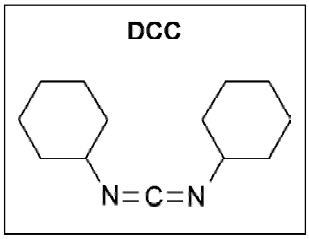

Figure 1. Dicyclohexylcarbodiimide (DCC).

seminars given in German, Khorana was reading papers in German and translating them to English. It was during this time that he came across a series of papers by Zetzsche and coworkers [4] on a class of chemical compounds called 'carbodiimides' that had been first discovered around 1870 but about which most organic chemists were unaware of. Khorana saw the potential power of this class of compounds in his future work. Indeed, one of these, dicyclohexyl carbodiimide (DCC) (*Figure* 1), would prove to be pivotal in much of his early years as an independent investigator for the synthesis of nucleotides and nucleotide coenzymes and in gene synthesis.

Back to India and then to Cambridge, England

After a year in Zurich in 1949, Khorana went back to India. But with the Indian subcontinent in disarray due to the partition of India, Khorana's family had been uprooted and was now living in the Punjab Province of India, and in spite of his PhD degree and postdoctoral research experience, Khorana could not find a job. Fortunately, Khorana had established a good friendship with George Kenner in Prelog's laboratory and Kenner had gone back to England and was working in the laboratory of Alexander Todd at Cambridge University. Khorana and Kenner were corresponding with each other and Khorana was offered a fellowship to work under Alexander Todd (a future Nobel Prize winner in Chemistry, in 1957). Todd was well known for his work on the chemistry of phosphates and nucleotides. In Todd's laboratory, Khorana and Kenner worked on peptides [5] and also obtained the first evidence for the potential use of DCC as a powerful reagent for activation of phosphate esters and the synthesis of nucleoside pyrophosphates [6]. In describing this result, Khorana says, "I remember that this simple method caused a great deal of excitement in the laboratory because the synthesis of pyrophosphates had been a central problem in Todd's laboratory and a very large amount of effort had been put into it."

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After three years in Cambridge, in 1952, Khorana was offered the position of an independent investigator at BCRC in Vancouver to

start his own research group. As to how he got the position, Khorana tells the story of Gordon Shrum, the Director of BCRC, and a physicist, who had come to UK and Europe to interview candidates for the position. He interviewed Khorana in Todd's office. After the interview, Shrum offered him the position but was quite candid in saying "you have all the freedom to do what you want", but that he could not offer much in terms of facilities. He also said that he wanted to hire an organic chemist because organic chemical research was the cheapest to carry out, "requiring only test tubes". Khorana was very excited at the opportunity to be starting his own research group; so, undaunted by what Shrum had told him, he accepted the offer, looked at the map to see where Vancouver was and moved there in 1952. Khorana often spoke highly of Shrum and was grateful to him for all the support that he provided during Khorana's stay in Vancouver and called him "an able and a visionary administrator".

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Years in Vancouver

Soon after arriving in Vancouver, Khorana was able to recruit a small staff to start his research program. He continued working with carbodiimides and started, in parallel, a program on chemical synthesis of sugar phosphates (mostly hexose and pentose phosphates) of biochemical interest. Many such sugar phosphates including ribose-1-phosphate, ribose 1, 5-diphosphate and 5-phosphoribosyl 1-pyrophosphate (PRPP) (*Figure* 2) involved in many important biochemical reactions, such as biosynthesis of the amino acids histidine and tryptophan, the nucleotides purine and pyrimidine, were synthesized chemically for the first time [7]

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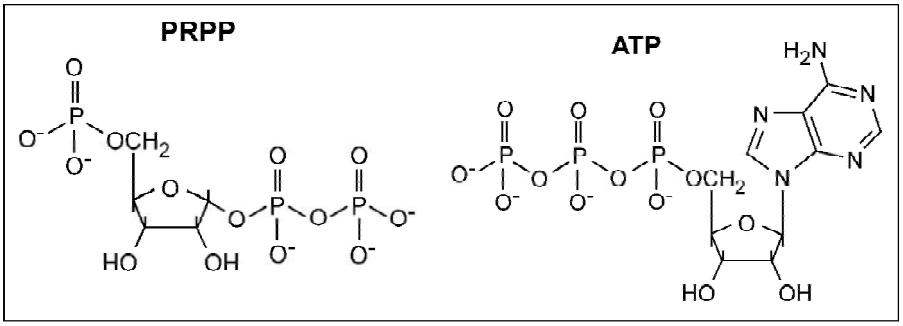

Figure 2. Phosphoribosyl pyrophosphate (PRPP) and adenosine triphosphate (ATP).

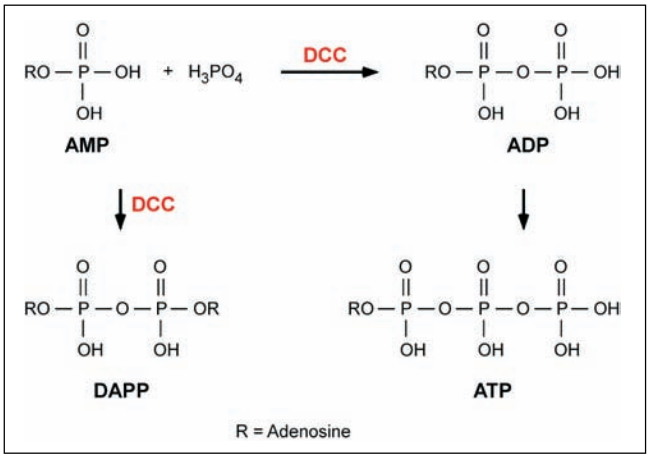

Figure 3. Synthesis of adenosine diphosphate (ADP), adenosine triphosphate (ATP) and P^1,P^2-diadenosine -5'-pyrophosphate (DAPP) from adenosine monophosphate (AMP) (modified from [8]).

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The use of carbodiimides as an extremely mild reagent for activation of phosphate esters led to the development of simplified one-step methods for synthesis of nucleoside polyphosphates [8], such as ribo- or deoxyribo- di- and triphosphates (ADP, ATP, etc.) (*Figure* 2), by reaction of a nucleoside 5-phosphate (for example AMP) with orthophosphoric acid in the presence of DCC (*Figure* 3). Formation of symmetrical dinucleoside 5'-5'-pyrophosphates, which occurred readily and in excellent yield when AMP was reacted with DCC alone was suppressed by the use of an excess of orthophosphoric acid.

Many of the nucleotide coenzymes known at the time such as NAD, FAD, coenzyme A (Co-A), UDP-glucose, etc., belong to a class of compounds called unsymmetrical nucleoside pyrophosphates (*Figure* 4), with newer ones being discovered in the 1950's. Khorana's finding that DCC could be used to readily

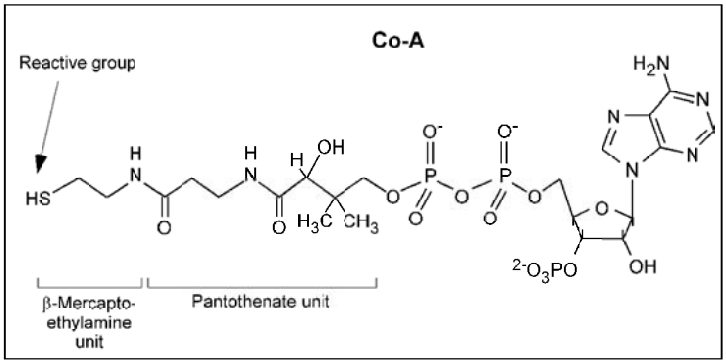

Figure 4. Coenzyme A (Co-A).

Figure 5. Synthesis of symmetrical and unsymmetrical pyrophosphates using DCC. pR, nucleoside 5'-phosphate. pR', phosphate monoester of an alcohol (for example, glucose phosphate, choline phosphate, etc.).

form pyrophosphate bonds stimulated others, in particular Eugene Kennedy, who was working on phospholipid biosynthesis to use DCC for the synthesis of UDP-choline, CDP-choline, ADP-choline and GDP-choline and to identify CDP-choline as the coenzyme involved in biosynthesis of lecithin [9].

Although DCC had proven very effective for the synthesis of nucleoside polyphosphates and nucleotide coenzymes, one of the side reactions was the formation of symmetrical pyrophosphates along with the formation of the desired nucleotide coenzymes, which are unsymmetrical pyrophosphates (*Figure* 5). Therefore, in a further improvement of the method, Khorana used DCC to convert AMP (or any nucleotide of interest) to a highly reactive derivative (AMP-5'-phosphoromorpholidate; *Figure* 6, top left), which would then react with phosphoric acid, pyrophosphoric acid or any phosphomonoester derivative to form nucleoside diphosphate, triphosphate or an unsymmetrical pyrophosphate. Development of this class of reagents led to even more effective

Development of this class of reagents led to even more effective syntheses of nucleoside polyphosphates and nucleotide coenzymes, culminating in the chemical synthesis for the first time of Co-A.

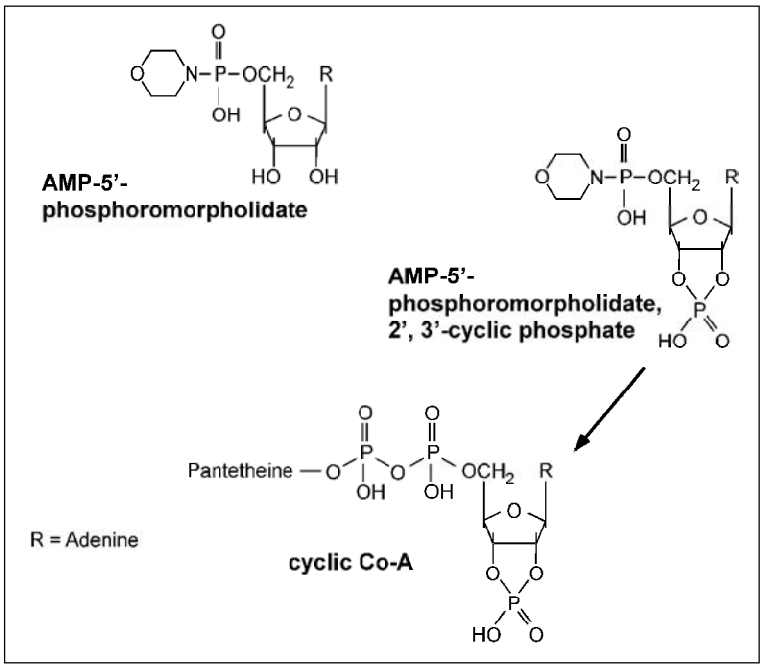

Figure 6. Steps leading to the synthesis of Co-A (modified from [10]).

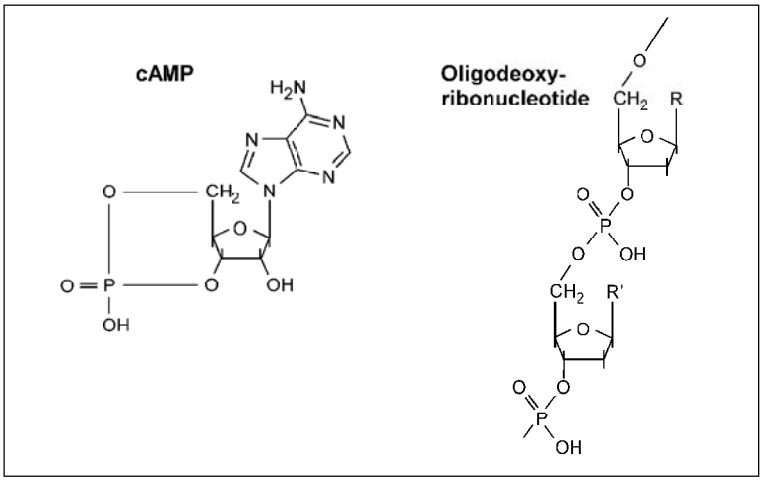

Figure 7. Cyclic adenosine monophosphate (cAMP) and oligodeoxyribonucleotide.

syntheses of nucleoside polyphosphates and nucleotide coenzymes, culminating in the chemical synthesis for the first time of Co-A [10] (*Figure* 6) and much improved synthesis of other coenzymes such as UDP-glucose, FAD, GDP-mannose, etc. [11].

The versatility of DCC as a reagent for the activation of phosphomonoester groups was also demonstrated by the synthesis of nucleoside 3'-5'-cyclic phosphates with DCC under highly dilute conditions [12]. Among the compounds synthesized were 3', 5'-cyclic AMP (cAMP) (*Figure* 7) and 3', 5' cyclic GMP (cGMP). These compounds were synthesized in 1960 soon after the discovery of cAMP by Sutherland and coworkers in 1958 [13] and named by them as a "second messenger" because of its important role in biological regulation. The discovery of cGMP as an equally important biological regulatory molecule in signal transduction came only years after its chemical synthesis by Khorana and coworkers.

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DCC and DNA Synthesis

In his quest for the development of methods for synthesis of genes, Khorana also used DCC for (i) the stepwise synthesis of deoxyribo-oligonucleotides [14], adding one nucleotide at a time (*Figure* 7) or (ii) polymerization of deoxy-nucleotides [15] to obtain homopolynucleotides of thymidine (*Figure* 8). This work

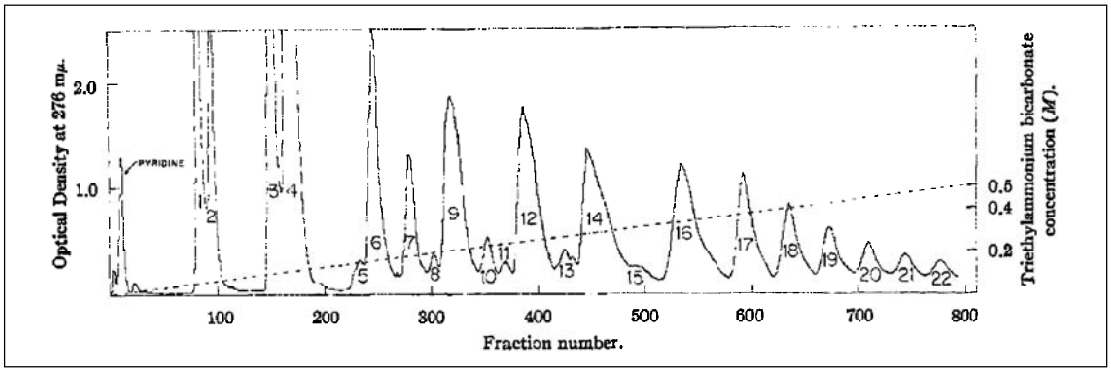

Figure 8. Chromatography of thymidine polynucleotides (total polymeric mixture) on DEAE cellulose (bicarbonate) column. Broken line shows triethylammonium bicarbonate gradient (from [15]).

initiated in Vancouver and the protecting groups used for protecting the sugar hydroxyls and the purine and pyrimidine ring nitrogen atoms would form the basis of work done in Wisconsin towards elucidation of the genetic code and chemical synthesis of deoxy ribo-oligonucleotides of specific sequence used in the assembly of a tRNA gene.

Khorana's Legacy

Khorana's synthesis of so many compounds of biological interest made them readily accessible for the first time and greatly facilitated biochemical experiments with them. Much of the work done in Vancouver was groundbreaking, up-to-date and of contemporary biological interest. This brought him to the attention of biochemists such as Arthur Kornberg, Paul Berg, Saul Roseman, Jerry Hurwitz, Leon Heppel, Fritz Lipmann, Charles Dekker, Herman Kalckar, Hans Boman, Roy Markham and others, many of whom visited his laboratory and spent summers working there. In addition to establishing life-long friendships between these scientists and Khorana and his group, which by then had grown substantially (*Figure* 9), these visits were of much benefit to the visitors and to the hosts. Paul Berg synthesized aminoacyl-adenylates [16] using DCC and took them to his laboratory for work on aminoacyl-tRNA synthetases. Arthur Kornberg synthesized deoxyribonucleoside-triphosphates [17] and used them for his work on DNA synthesis. In turn, Khorana and his group learnt much about biochemistry, enzymology and biology in general from seminars and discussions with the visitors. It also was

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Figure 9. The Khorana group in Vancouver, 1959, including summer visitors: John Vizsolyi, Hans Boman (from Uppsala), Roberts Smith (from UCLA), Irving Goldberg (from Rockefeller University), Bernhard Lerch, John Moffatt, Gordon Tener, Herman Kalckar (Denmark, USA), Bill Razzell, and Michael Smith.

probably a turning point for Khorana, who was getting more and more interested in biology. He was now convinced that the future of synthetic organic chemistry, at least for him, lay in applying it to fundamental problems in biology. He followed this course and became a pioneer in the field of chemical biology.

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Har Gobind Khorana believed sincerely in Otto Loewi's motto "We must be modest except in our aims". In science, he aimed high, working towards goals that seemed unreachable at the beginning. Most remarkably, in spite of his towering accomplishments in science at Vancouver, Wisconsin and MIT, he remained a most humble and a modest person, easily approachable to a young student or to his peers. Over the years he won many awards and prizes and accolades. What he appreciated most were scientific meetings held in his honor at various universities by his former students, including the one at Vancouver in 1993 (*Figure* 10). He felt gratified to have a park named after him at the University of British Columbia campus in Vancouver (*Figure* 11) and an auditorium in the Department of Biochemistry at Wiscon-

Figure 10 (left). Vancouver 1993. H Gobind Khorana with Arthur Kornberg and Paul Berg.

Figure 11 (right). Khorana Memorial Park at the University of British Columbia in Vancouver.

Figure 12. Khorana with the author outside the Biology Department building at MIT in 2002. .

sin. He also appreciated very much the naming of an adjoining park in Vancouver for Michael Smith, one of his former postdoctoral associates at Vancouver and at Wisconsin, and a Nobel Prize winner in Chemistry in 1993.

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Suggested Reading

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