Profile of Masayori Inouye

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Masayori Inouye, a distinguished professor at the Rutgers Robert Wood Johnson Medical School, has cut a wide swath in biochemistry and molecular biology. His wide-ranging accomplishments include the determination of genetic codons in a protein and thus, for the first time, a partial DNA sequence of a gene, elucidation of the mechanism of biogenesis and assembly of membrane proteins, and creation of a protein-synthesizing bioreactor, among others. Many of his findings hold promise for application in biotechnology and clinical medicine. Inouye, who was elected to the National Academy of Sciences in 2019, is now trying to determine how genetic codons first evolved. Inouye's Inaugural Article (1) presents his hypothesis that primordial proteins consisted of seven amino acids, which he is using to synthesize an enzyme that may have been fundamental to the origin of life.

Masayori Inouye. Image credit: Colin Germain (Rutgers University, Piscataway, NJ).

Education and World War II

Inouye was born in Port Arthur, Manchuria, which is now known as the Lüshunkou District of Dalian, China. His father, who held a PhD in organic chemistry, along with his mother, moved to Port Arthur from Japan as an officer of the Japanese government. His father died seven years later, months before Japan's attack on Pearl Harbor that precipitated United States engagement in World War II. The Soviet Invasion of Manchuria followed in 1945, causing further hardship to Inouye's family. Local schools closed, preventing him from continuing his studies until 1947, when he, his mother, and two siblings moved to Japan.

Despite the challenges, Inouye says he always knew he would be a scientist. He says, "My father's background strongly influenced me, and in Japan then, the eldest son was expected to inherit the occupation of his father." Inouye attended Osaka University, where he earned a Bachelor's degree in chemistry and Master's and PhD degrees in biochemistry. From 1963 to 1968, he served as an instructor in the university's Department of Molecular Genetics and did postdoctoral work in the laboratory of molecular biologist Akira Tsugita.

First Partial DNA Sequence

During his postdoctoral stint, Inouye collaborated with Tsugita and phage geneticist George Streisinger to determine the codons of a gene, T4 phage lysozyme (2, 3). The approach involved comparing the amino acid sequences of several double frameshift mutants with those of the wild-type lysozyme. Inouye says, "I established a method for large-scale purification by designing and building a 600-liter culture tank." The innovation, combined with traditional methods for protein structure analysis, allowed the researchers to determine the first known partial nucleotide sequence of a gene, marking a first in DNA sequencing.

Inouye, married and then with two children, moved with his family to the United States in 1968 to accept a research associate position in the Princeton University Department of Biochemistry. There, he was mentored by Arthur Pardee, who provided career guidance and encouraged him to stay in the United States even when tempting opportunities arose in Japan. "Pardee was a very generous person," Inouye says. "He trusted my science, gave me recognition, and allowed me to work independently."

Molecular Biology of Membrane Biogenesis

After 2 years at Princeton, acting on the advice of biochemist Bruce Alberts, Inouye accepted an associate professorship in biochemistry at the State University of New York at Stony Brook in 1971. He later

This is a Profile of a member of the National Academy of Sciences to accompany the member's Inaugural Article on page 28572.

advanced through the ranks to become professor and Chairman of the Department of Biochemistry in 1981.

Inouye maintained an active research program, initiating work on the mechanisms of membrane cell division in the model organism *Escherichia coli*. After isolating an *E. coli* messenger RNA (mRNA) (4), Inouye and his colleagues determined its sequence of 322 bases, the first mRNA sequence to be determined (5).

Insights on Membrane Signal Transduction

Since 1970, Inouye has conducted formative research on the structure and function of *E. coli* membrane proteins. He and his team demonstrated the role of signal peptides for protein secretion across the membrane (6). Inouye says, "The study marked the first time that oligonucleotide-directed, site-specific mutagenesis was applied to alter the amino acid sequences of a protein to create mutant proteins." He and his colleagues additionally engineered a chimeric signal transducer to activate *E. coli* membrane pore gene expression in response to the enzyme aspartate (7).

After over a decade of work at Stony Brook, in 1987 Inouye became Chair of the Department of Biochemistry at the Robert Wood Johnson Medical School of Rutgers University, where he has since stayed. Continuing his research on signal transduction, Inouye and his colleagues collaborated with medical biophysicist Mitsuhiko Ikura of the University of Toronto. They revealed via NMR the 3D structure of the kinase domain of a transmembrane protein and its role in signal transduction across the membrane (8).

Discovery of Antisense RNA

Inouye has mentored numerous graduate students and postdoctorates, with many becoming leading scientists. For example, a postdoctorate in Inouye's laboratory, Takeshi Mizuno, now a professor at Nagoya University, theorized in 1984 that when short, noncoding RNA is produced at high osmolarity, it might function as a gene repressor for an *E. coli* outer membrane protein. Mizuno, Inouye, and colleague Min-Yuan Chou tested the theory and reported a proof-of-concept (9).

The finding marked the first discovery of an RNA repressor for gene expression, now commonly termed antisense RNA. Mizuno mischievously called it micRNA (mRNA-interfering cRNA), which reflected its small size and the first letters of the researchers' surnames. It would take another 14 years before RNA interference gained wider attention in the scientific community due to the work of biologists Andrew Fire and Craig Mello, who won a 2006 Nobel Prize for their work in this field.

Demonstrating the discovery's clinical potential, Inouye and colleagues created cells that produced antisense RNAs, which conferred immunity against bacteriophage infection (10). For this and other achievements, Inouye was elected as a member of the American Academy of Arts and Sciences in 1986. Subsequently, he received a National Institutes of Health Merit Award in 1990, and other honors. Antisense RNAs as well as antisense oligonucleotides continue to be widely tested for clinical applications.

Discovery of mRNA Interferases

Twenty years after the discovery of antisense RNA, Inouye's team serendipitously discovered a second mechanism for inhibiting protein synthesis in cells (11). While characterizing an *E. coli* protein, they identified the gene *mazF*, which cleaves mRNAs at a specific sequence, ACA. Inouye observed that upon expression of the *mazF* gene, cell growth was inhibited yet metabolic activities remained unaffected.

With the gene *mazE*, *mazF* is an operon encoding a toxin and *mazE* an antitoxin in *E. coli*, such that the unit of linked genes is an ACA-specific mRNA interferase enzyme, which is responsible for growth arrest and cell death. Inouye and coauthor Hirofumi Nariya later found a homolog of this toxin–antitoxin system required for fruiting body formation in the soil bacterium *Myxococcus xanthus* (12). With colleague Yoshihiro Yamaguchi, Inouye reported that *E. coli* contains at least 37 toxin–antitoxin systems that target various cellular functions (13). The authors noted the potential of these systems for use in medical research, molecular biology, and biotechnology. Inouye and his team further showed that mRNA interferases may have a potential role in anti-HIV gene therapy (14).

They also identified an mRNA-cleaving analogous toxin in *Bacillus subtilis* (15), the X-ray structure of which was determined. Inouye and his colleagues then found the homolog MazF-hw in the archaea *Holoquadra walsbyi* from a Sinai Peninsula hypersaline pool (16).

Single-Protein Production System

Because MazF expression in *E. coli* removes nearly all mRNAs, Inouye and his team were able to transform cells of the bacterium to a bioreactor that produced only a select protein (17). They termed their creation the "single-protein production" (SPP) system. Inouye says, "Using the SPP system, a protein of interest can be isotope-labeled without labeling any other cellular proteins."

As a result, Inouye and other researchers may use the system in conjunction with NMR to study protein structure and dynamics in living cells. With his colleagues, Inouye recently used the technology to monitor intracellular conditions of *E. coli* (18). The researchers found that the growth phase of the host cells affects the detectability and resolution of the NMR spectra.

Evolution of the Genetic Code

Inouye is currently studying how genetic codons may have evolved when life originated. Inouye's theory, presented in his Inaugural Article (1), holds that because living organisms are composed of proteins encoded by the same genetic codons, all forms of life likely derived from a single common ancestor. It also holds that the present genetic code consisting of 61 codons encoding 20 amino acids evolved in a stepwise manner. This predicts that there was a set of primordial amino acids.

Inouye hypothesizes that two classes of codons for serine emerged at different times and that the primordial proteins consisted of seven amino acids: Glycine, alanine, aspartic acid, glutamic acid, serine, valine, and arginine. Inouye says, "On the basis of this hypothesis, I am currently synthesizing an enzyme consisting of only these primordial amino acids. This approach will elucidate the validity of my hypothesis."

Considering the breadth of his accomplishments, Inouye says, "I feel that I am very fortunate, as I am still able to work at the bench."

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