BIOGRAPHICAL MEMOIRS

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Hirohei Yamamura and Shun-Ichi Nakamura

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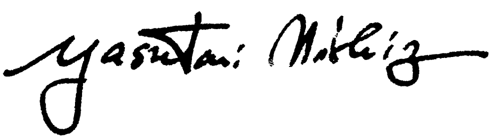

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BACKGROUND AND EARLY LIFE

Yasutomi Nizhizuka was born in Seido Village, Muko-gun (now City of Ashiya), Hyogo-Prefecture, on 12 July 1932, as the second son of Yasunobu and Nobuko Nishizuka. His elder brother, Yasuaki (1922–95, pathologist, President of Aichi Cancer Center), was 10 years old, his eldest sister, Hiroko, was aged 8, his elder sister, Fumiko, was 5, and he was a heterozygotic twin with his sister Junko. His mother, Nobuko, was from the Ijichi family, a huge landlord, which traced its roots back to a powerful family of the Heian Era (eleventh century). When Yasutomi was an infant, he was so weak that his parents were not sure that he would survive to adulthood. He had intussusception (ileus) when he was six months old. He barely survived after emergency surgery at Daido Hospital in Osaka. Since then, he frequently suffered from diseases associated with stomach ache. He was always weak and ailing.

In April 1933, his father Yasunobu (1892–1959) was appointed Professor of Obstetrics and Gynaecology at Manchuria Medical University of Mukden (now Shenyang, China) and the whole family moved from Kobe to Mukden via Dalian. Yasutomi spent his infancy there. In April 1936, Yasunobu resigned from his job and took his family from Mukden via Pyongyang and Gyeongseong to Pusan by land, and then to Japan by sea. The family again settled in Ashiya, and Yasunobu began working for Kitano Hospital in Osaka.

In April 1937, Yasunobu became Chairman of Obstetrics and Gynaecology at Nagoya Municipal Hospital and the family moved to Nagoya City. Japan was at war with China

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(Nisshi Jihen) and the social conditions began to deteriorate. In April 1939, Yasutomi entered an elementary school but he was a weak boy and was absent from school almost throughout the first semester. He was quiet and small and inconspicuous during his elementary school days. It was wartime and children had few toys to play with. In summer, Yasutomi would enjoy catching cicadas. In fact he was very good at capturing cicadas. Insects and other wild creatures were good friends for him to play with in his childhood. It was a time when children were not allowed to swim in the sea. Instead, he would swim in dangerous neighbourhood rivers with friends and was often scolded by his mother. In March 1945, one day before the graduation ceremony, his elementary school was burned in an air raid. The graduation ceremony was performed without diplomas for the pupils.

In April, 1945 Yasutomi entered Showa Junior High School. He was still small and weak. His high school was located far from his home and there was no bus service. Yasutomi had to walk to school. He was always pale and would become feverish as a result of fatigue on Saturdays. During the summer vacation, though, he became an active boy. Here is an episode which demonstrates that he was no longer weak but had become an active boy. He enjoyed walking into a muddy river to catch two bucketfuls of carp to carry home, one in each hand. People were starving in those days and everybody was happy with the feast. He was fond of making things and was always making something at home. All food was rationed at that time. When powdered sweet potato was distributed, Yasutomi made a wooden box and passed an electric current through the potato to make steamed bread for his family to enjoy instead of rice. He was a very clever boy and full of ideas. When he entered senior high school he became taller and stronger and more serious about his studies. His favorite subject was geometry. In April 1948, his father became Professor of Obstetrics and Gynaecology at Nagoya Women's University.

Undergraduate studies at Kyoto University

In April 1951, he entered Kyoto University. He lodged at his relative's house in Kyoto. After he entered the university he learned to play the piano all by himself and it became his habit to play whenever he came home from Kyoto. His sister was living nearby and he entertained his nieces with his piano playing. There was always laughter around him. He told, keeping a straight face, many stories that people could not distinguish between fact and fiction. There are many episodes showing his kindness to his parents. When he came home during winter vacation he cut firewood or charcoal for his mother. His family never found him to get angry at home. He pleased his father when he built a henhouse for him. He would groom his father's garden and care for carp in the pond. He made wire netting to cover the pond to protect the carp. He was good at drawing and, using coloured pencils, illustrated a poem that his cousin had written.

In April 1953, he entered the School of Medicine, Kyoto University. Then he became a man of scholarship and it was as though his studies were his hobbies, too. It is especially noteworthy that Yasutomi made perfect notes of neuroanatomy lectures by Professor Ko Hirasawa, in which figures of nerve conduction were beautifully drawn with coloured pencils. He handmade a book combining several notebooks that he treasured. It was superior to the books of anatomy on sale at the time. Yasutomi gave his heartfelt respect to Professor Hirasawa. He told his sisters that he wanted to perform a study that would win him the Nobel Prize. He played tennis and enjoyed ice-skating.

In March 1957 he graduated from the School of Medicine, Kyoto University, became an intern at the University Hospital and passed the board examination in the following year. He studied very hard for the entrance examination for the Department of Medical Chemistry, Graduate School of Medicine, Kyoto University. He made a metabolism map all by himself and learned all of it by heart. Although his brother suggested pathology, Yasutomi believed that the days of morphology were over and it was going to become the age of biochemistry.

Road to biochemistry: Department of Medical Chemistry, Kyoto University

In April 1958, Yasutomi joined, as a graduate student, the laboratory of Professor Osamu Hayaishi, who had come back to Japan from the USA after his great discovery of oxygenase. He wrote his first paper on transaminase from *Pseudomonas aeruginosa* (1)*. The first paper that made him famous was a study that enzymatically clarified the synthesis of NAD^+ from tryptophan in rat liver (2). This paper won him an award from the Japanese Biochemical Society when he was 31 years old. In the same year (1963), in May, Yasutomi married Miss Sachiko Mano. In July 1964, he won an NIH fellowship and decided to visit Professor F. Lipmann (ForMemRS 1962) at Rockefeller University. He left his newlywed wife in Japan and went to the USA. His first daughter was born in February 1965, while he was abroad. Yasutomi did not see his first baby, Yukiko (now Mrs Tanaka, the wife of a high-ranking official of the Ministry of Home Affairs of the Japanese Government) until she was five months old, in July 1965. The only thing that he could do for his baby as a father was to send her a baby buggy from the USA.

It was a period when the mechanism of protein synthesis was being investigated. Yasutomi succeeded in isolating two protein elongation factors (related to GTP) under the guidance of Professor Lipmann. They were named T factor (later separated to Tu and Ts) and G factor (GTPase) (3). It was a very important finding for clarifying the mechanism of protein synthesis. He then returned to Professor Hayaishi's laboratory and published several papers with Dr T. Honjo, Dr K. Ueda, Dr A. Ichiyama and others, using NAD^+ as a substrate on protein mono- and poly-ADP-ribosyl transferase reactions and showed that when protein elongation factor was ADP-ribosylated by diphtheria toxin it lost its function (4, 5).

In the autumn of 1968, Dr Nishizuka began his studies on protein phosphorylation. This was a little before Professor E. G. Krebs and his colleagues found cyclic-AMP-dependent protein kinase (PKA). He understood that phosphorylation of nuclear proteins had been a research theme of many Rockefeller scholars since the time that the Rockefeller Institute was founded. Back home, it was a time when students' protests against university authorities became violent—almost leading to bloodshed—and their call for structural reform was voiced loudly all over Japan; Kyoto University was no exception. Students would shout about shutting down universities. At that time Dr Nishizuka was chosen as the first Professor of the Department of Biochemistry II, Kobe University School of Medicine. On 1 January 1969 he moved to Kobe.

* Numbers in this form refer to the bibliography at the end of the text.

Outstanding biochemist: Department of Biochemistry, Kobe University School of Medicine

Professor Nishizuka's new laboratory at Kobe University was poorly equipped for a new professor. He was provided with laboratory space in a corner of the student teaching laboratory for social medicine. His corner was separated from the main teaching laboratory only by bookshelves. The Department of Biochemistry II had formerly been a department of occupational medicine under Professor Kazuo Furusawa, famous for occupational hygiene. It was equipped with old instruments for physiology but none for biochemistry. There were many remodeled bicycles for studies on energy metabolism, and the facilities included a bathroom. Professor Nishizuka was unable to perform any biochemical experiments. He redesigned all of the space, using the funds provided by the university to a new professor, to establish a new department. The first members of this department were M. Takeda, lecturer, A. Kumon, research associate, and H. Yamamura, a graduate student. In the autumn of the same year, the laboratory and a cold room were completed and the research activities began at last. Studies on liver protein kinase were continued in Kobe. The laboratory environment was miserable as far as equipment was concerned. There was no high-speed centrifuge or fraction collector. All one could expect was manpower and strong determination. Fellows were overwhelmed with joy when these pieces of equipment were installed. No research grant from the Ministry of Education was given to Professor Nishizuka's group for that first year, and he alone was adopted as a member of a specific research area, which disappointed him. Professor Nishizuka was not discouraged; he told himself that it was all right because at least one of his research proposals had been approved and funded.

His first studies at Kobe investigated how protein phosphorylation mediated hormone actions. His group had isolated a functionally undefined kinase from rat liver, using calf thymus histone as a phosphate acceptor, and confirmed that cyclic AMP greatly stimulated its catalytic activity. Soon Professor Nishizuka's colleagues observed that when the enzyme preparation was subjected to column chromatography, two enzyme peaks, cyclic-AMP-dependent and cyclic-AMP-independent, appeared, although the two peaks showed otherwise identical catalytic properties (6). It was noted that cyclic AMP converted the former form to the latter. Before long, in 1970, four individual laboratories—those of Krebs, Lipmann, Gordon Gill and Nishizuka—simultaneously reported that PKA consists of catalytic and regulatory subunits, and that cyclic AMP activates the enzyme by dissociating these subunits (7). Professor Nishizuka seemed very nervous in writing his first paper from Kobe. He must have had a feeling that these two publications were going to make him compete against the world from Kobe. He was devoted to the field of cyclic AMP in those days.

His lectures were very interesting and attracted young students. He would tell his students, 'the University of Tokyo and Kyoto University may have won reputations as leading organizations of Japan in the field of science. Believe me, Kobe University can and will be a leading university, just like Tokyo and Kyoto'. Many of those students who listened to him came to his laboratory. Dr Y. Takai (now a professor at Osaka University) was one of those first students who visited his laboratory when he had no classes. Many outstanding students came to join his laboratory.

From 1974, Professor Nishizuka's interest shifted from cyclic AMP to cyclic GMP. It was considered that cyclic GMP would function as a second messenger just as cyclic AMP does. Although his group had purified cyclic-GMP-dependent protein kinase (PKG) from rat

tissues, there was very much less PKG than PKA in mammalian tissues, and no specific catalytic activity of PKG could be found (8). Furthermore, PKG, unlike PKA, was a single polypeptide chain and was activated by binding cyclic GMP at a regulatory region within it. An inhibitory interaction between catalytic and regulatory regions of the PKG molecule was suggested because limited proteolysis with trypsin or calpain generated a constitutively active fragment of the enzyme that was no longer sensitive to cyclic GMP. This was a key observation that led to Professor Nishizuka's subsequent finding of a new protein kinase. Now, his interest shifted to calcium.

Discovery of calcium-activated, phospholipid-dependent protein kinase (protein kinase C, PKC)

During analyses of mammalian PKG it had been noticed that rat brains that had been stored frozen contained a very active protein kinase whose activity was independent of any cyclic nucleotide. The only requirement for this enzyme was Mg^{2+} (protein kinase M; PKM). Working long and hard in the cold room, the Nishizuka team found that much higher levels of this novel kinase could be purified from stocks of frozen brains than from the brains of freshly killed rats. In addition, yields improved even more when, for economy, protease inhibitor levels were reduced. Professor Nishizuka then drew on his experience with PKG and hypothesized that they were studying a constitutively active proteolytic fragment that had lost its auto-inhibition, and set out to identify the 'pro-enzyme' and the activators that conferred phosphotransferase activity (9).

The Nishizuka group identified the pro-enzyme and reported that it was activated by membrane phospholipids, in particular phosphatidylserine. The group soon noticed that crude extracts of phospholipids from brains, rather than pure lipids, were the most effective at activating PKC and set out to uncover the nature of the 'impurity' that activated PKC. The activating component was diacylglycerol (10), leading Nishizuka to make the conceptual breakthrough that PKC might be the target for one of the products of the lipid hydrolysis pathway (11) discovered by Hokin & Hokin (1953).

To obtain conclusive evidence for diacylglycerol as the physiological mediator of hormone action, a method was needed to activate PKC by introducing this neutral lipid into intact cells. Natural diacylglycerols that have two long fatty-acid chains could not readily be intercalated into the cell membrane. He found that if one of the fatty-acid chains was replaced with an acetyl group, the resulting diacylglycerol such as 1-oleoyl-2-acetylglycerol (OAG) was sufficiently soluble in water to allow it to be dispersed into the membrane lipid bilayer, where it activated PKC directly.

In the meantime, several reports in the literature had suggested an excellent experimental cell system. Susan Rittenhouse-Simmons at Harvard showed that diacylglycerol accumulated transiently in thrombin-stimulated platelets, possibly as a result of inositol phospholipid hydrolysis. In contrast, Phil Majerus in St Louis and Dick Haslam in Hamilton, Ontario, independently reported that on stimulation of platelets with thrombin, two endogenous proteins of molecular masses of 20 and 47 kDa were heavily phosphorylated. It was found that the 20 kDa protein was myosin light chain that was phosphorylated by a specific calmodulin-dependent kinase in response to an increase in the intracellular Ca^{2+} concentration. In contrast, the enzyme responsible for phosphorylation of the 47 kDa protein had not been identified.

The Nishizuka group found that the 47 kDa protein was phosphorylated by PKC on stimulation of platelets by thrombin. The addition of the membrane-permeant diacylglycerol OAG to platelets induced the phosphorylation of the 47 kDa protein (12). This protein was shown to be a specific PKC substrate, later called pleckstrin. Thus, the 47 kDa protein served as an excellent endogenous marker for the increase in the diacylglycerol-dependent activation of PKC. In the spring of 1983, the Nishizuka group had shown that both an increase in Ca^{2+} and PKC activation were essential and acted synergistically for the full activation of platelets to release serotonin (14). At this time they used a Ca^{2+} ionophore to increase intracellular concentrations of this cation and did not know where Ca^{2+} came from physiologically, although Bob Michell (FRS 1986) had postulated that inositol phospholipid hydrolysis could open a Ca^{2+} gate (Michell 1975). In the autumn of 1983, M. J. (later Sir Michael) Berridge (FRS 1984) and his colleagues in Cambridge presented evidence that inositol 1,4,5-trisphosphate, the other product of the inositol phospholipid hydrolysis, could mobilize Ca^{2+} from its internal stores (Streb *et al*. 1983). The traditional pathway of signal flow from the cell surface into the cell interior through PKC activation and Ca^{2+} mobilization emerged in this way in the early 1980s.

It was well known that croton oil causes a marked augmentation of carcinogenesis when applied at weekly intervals to the skin of mice in conjunction with a very dilute solution of benzpyrene in acetone. The oil was obtained from the seeds of croton and contained phorbol ester, a powerful tumour promoter. When applied to the cell, it elicited a wide variety of responses that were similar to those of hormones. Several kinetic studies with various cell types had suggested that the primary site of its action could be its own receptor located on cell-surface membranes.

At first Professor Nishizuka expected that phorbol ester would produce diacylglycerol to activate PKC, and mimic the thrombin action. However, it became clear that diacylglycerol was not the messenger for the phorbol ester-mediated PKC activation. He noticed that the phorbol ester contained a diacylglycerol-like structure in its molecule that was very similar to the membrane-permeant diacylglycerol, OAG, that they used routinely. It was possible to imagine that phorbol ester would activate PKC directly just as its receptor. A series of experiments showed that phorbol ester mimicked the action of diacylglycerol and activated PKC directly, eventually leading to cellular responses (13). The results were confirmed immediately by the scientific community and in the following year several groups of investigators showed that PKC and the phorbol ester receptor could be co-purified, and they found stoichiometric binding of phorbol ester to PKC, using the enzyme in a pure form. As a result, the traditional concept of tumour promotion originally proposed by I. Berenblum from Oxford had been replaced by an explicit biochemical explanation that centred on understanding the role of PKC.

Along this line of study, phorbol esters and membrane-permeant diacylglycerols have been used as crucial tools for the manipulation of PKC in intact cells and have allowed the wide range of cellular processes regulated by this enzyme to be determined (15). (This paper was the most frequently cited paper in the world in the 1980s.)

Professor Nishizuka's discovery of PKC and the action of phorbol ester in the early 1980s definitively established a role for PKC in cellular signal transduction, and made one of the greatest contributions in physiology and medicine in these decades (16). (This paper was the most frequently cited paper published in 1986.) Today it is known that the mammalian PKC is a large family of serine/threonine protein kinases consisting of ten isotypes encoded by nine genes (17).

His attitude as a researcher

Professor Nishizuka studied tryptophan metabolism, protein synthesis, ADP-ribosylation and protein phosphorylation. He challenged the most important themes of the times and always led his field. He evaluated scientific quality in terms of physiological relevance, he valued creativity and paid no heed to research that pursued popularity. His great achievements were the result of his outstanding insights, charisma that attracted followers and his indomitable spirit. He always did his best. He gave the same serious attention to writing his English articles as his Japanese papers; to his lectures at international gatherings and even to informal talks at small group discussions. He made himself perform to the best of his capabilities.

President of Kobe University

Kobe University suffered great damage from the Hanshin Awaji earthquake in January 1995. Professor Nishizuka was appointed President of Kobe University just after the earthquake. He literally forwent his sleeping and eating time and led the Kobe University staff in the restoration of the university. He successfully achieved this mission. He founded the Biosignal Research Center at Kobe University, Rokko Campus, and tried to make it a 'Mecca' for research on signal transduction. Beside a very demanding job as President he mentored young researchers and devoted himself to the development of the Center. Until he retired from the presidency in February 2001 he dedicated six years to the development of Kobe University. Although Kobe University unfortunately experienced the terrible earthquake in 1995, the only consolation was that Professor Nishizuka was the President of Kobe University and the university was restored to an even a better condition than could have been imagined. Professor Nishizuka's aim as President was to educate good brains and to produce leading young scientists. He believed that youth is always a key to the development of science and also plays a leading role in the evolution of university. He hoped that the students of Kobe University would have the confidence to compete with world authorities and leave influential footsteps in every field that will be followed by the next generation.

Retirement

After he retired from Kobe University, Professor Nishizuka became the President of Hyogo Prefecture Adult Disease Center, as well as an advisor to the members of the administration of Hyogo Prefecture; he contributed to the development of medicine and academy in Hyogo Prefecture. He also undertook the role he was offered as an honorary adviser by Sysmex Corporation, a health care testing company founded in Kobe. He stimulated young researchers of their laboratory, while he himself enjoyed intense discussions with them. He took another appointment as an advisor to the Japan Society for the Promotion of Science and dedicated himself to the development of science in Japan.

Because his house was destroyed by the Hanshin Awaji earthquake, his younger daughter, Tsugiko, a first-rate licensed architect, designed for him a new house that perfectly matched his wishes. He was able to spend his later years in a lovely new house, meeting a variety of people.

Professor Nishizuka never failed to pay close and careful consideration to anything he did or any problem that befell his colleagues or students. He always kept his warm eyes on anybody in trouble. When he heard that any of his acquaintances had fallen ill, he gave full personal attention to the matter and introduced them to the best doctor. He gave special consideration to his own health, which he had checked regularly. In 2001, he was hospitalized for a mild brain infarction for about two weeks after he experienced light numbness in his hand. After that, he was again hospitalized in the ophthalmology department for retinal artery infarction. Aside from these episodes, he was generally in good health. Whenever he visited Kobe University Hospital for his regular health check, he also visited our laboratory and told his former colleagues that he was fine. But he seemed tired, telling them, 'I think I should resign all my assignments next year. I am tired.' Regardless of the lab results, which showed there were no problems, he probably knew more about his own health. One month before he passed away, Professor Nishizuka came to the medical campus to give a lecture to postgraduate students. The alumni hall in the campus was filled to capacity and he spoke for one and half hours. It was a truly wonderful and memorable talk. It turned out to be his last lecture.

We were very fortunate to become his students and privileged to have his guidance for many years. Professor Nishizuka recommended that we should study for years under the world's leading scientists such as M. Rodbell, E. G. Krebs, J. L. Goldstein ForMemRS and G. Blobel, who later won the Nobel Prize. Nothing is more fortunate than such an opportunity for anybody who wishes to carry out studies in medical sciences. We have no words adequate to thank Professor Nishizuka. We know that our duty is to convey what we have learned from Professor Nishizuka to a younger generation. We thank Professor Nishizuka for all the lessons he taught us and, last but not least, we express our heartfelt condolences.

Awards and prizes

1986 The Award of the Japan Academy
1987 Cultural Merit Prize, from the Japanese Government
1988 The Alfred P. Sloan Jr Prize, from the General Motors Cancer Research Foundation, USA)
Gairdner Foundation International Award, Canada
The Order of Culture, from the Emperor of Japan
1989 The Albert Lasker Basic Medical Research Award, from the Albert and Mary Lasker Foundation, USA
1992 Kyoto Prize in Basic Sciences, from the Inamori Foundation, Japan
1994 The Dale Medal, from the British Endocrine Societies
1995 The Wolf Prize in Medicine, from the Wolf Foundation, Israel
The Jimenez Diaz Award, from the Foundation Jimenez Diaz, Spain
The Schering Prize, from the Ernst Schering Research Foundation, Germany
1996 The Banerjee Medal, from the Asiatic Society, India

Academy membership

1988 Foreign Associate of the National Academy of Sciences, USA
1990 Foreign Member of the Royal Society, UK

1991 Member of the Japan Academy, Japan
1992 Foreign Associate of l'Académie des Sciences, France
Foreign Honorary Member of the American Academy of Arts and Sciences, USA
1993 Foreign Member of the Real Academia de Ciencias, Spain
1994 Foreign Member of the Deutsche Akademie der Naturforscher Leopoldina, Germany
1995 Honorary Fellow of the Asiatic Society, Calcutta, India
1998 Associate Fellow of the Third World Academy of Sciences, Italy

Named award lectures

1979 The 1st Fritz Lipmann Lecture, Paris-Grignon, France
1984 The 3rd Smith-Klein Lecture, Michigan University, USA
The Emory Commencement Lecture, Emory University, USA
The 10th Otto Kreyer Lecture, Harvard University, USA
1985 The 28th Philip Schaefer Lecture, Washington University, St Louis, USA
The 3rd Miles Bayer Lecture, Yale University, USA
The 3rd Geevers Lecture, Illinois University, USA
1987 The 10th Chilton Lecture, University of Texas, USA
The 5th Hans Lindner Lecture, Weizmann Institute, Israel
1990 The Neuroscience Distinguished Lecture, Louisiana State University, USA
The 6th Tairner Lecture, Stanford University, USA
1991 The Slater IUB Congress Plenary Lecture, Jerusalem, Israel
1992 The 5th Edwin Krebs Lecture, Washington University, Seattle, USA
The 2nd Bloemendal Lecture, Universiteit Nijmegen, The Netherlands
1993 The 5th Durham Lecture, Duke University, USA
The 8th Ray & Robert Kroc Lecture, Harvard University, USA
1994 Northwestern Commencement Lecture, Northwestern University, USA
1995 The 27th Jimenez Diaz Memorial Lecture, Universidad Complutense, Spain
1996 The 4th Banerjee Lecture, Calcutta University, India

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The frontispiece photograph was taken in 1991 and was found in his belongings. The photographer is unknown. It is reproduced with permission from Mrs Nishizuka.

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(2) 1963 (With O. Hayaishi) Studies on the biosynthesis of nicotinamide adenine dinucleotide. I. Enzymic synthesis of niacin ribonucleotides from 3-hydroxyanthranic acid in mammalian tissues. *J. Biol. Chem.* **238**, 3369–3377.

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