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In Memoriam John J. Holland (1929-2013): a Pioneer in Molecular Virology

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John J. Holland, Professor Emeritus of Biological Sciences at the University of California, San Diego (UCSD), died on 11 October 2013 after a short illness. John was a pioneer in RNA virology, changing the way we think about RNA virus evolution. He was a scientific leader who served the virology community at the international, national, and university levels. John was an outstanding teacher and mentor who will be greatly missed. We thank John's many friends, colleagues, trainees, and family for contributing their remembrances of this remarkable scientist.

John J. Holland 1929–2013

John died in Mesquite, Nevada, where he had enjoyed retirement with Dottie, his wife of more than 50 years. Prior to retirement, John was a Professor in the Department of Biology at UCSD and a member of the University's Center for Molecular Genetics. Professionally, John was an outstanding leader in virology research and teaching, establishing paradigms in RNA virus molecular biology, persistence, and evolution. Personally, he had a zest for life and an intense interest in the professional and personal success of his scientific colleagues, students, and postdoctoral fellows. John is remembered for his tremendous intellect, integrity, and rigorous science and the way in which he infused enthusiasm, joy, and laughter into the pursuit of excellence. His humility and self-effacing nature would cause him, if he were still alive, to wave this all off with a loud, irreverent comment and a cackle of laughter.

John Holland was born on 16 November 1929 in Pittsburgh, Pennsylvania. He graduated from Loyola University in Los Angeles (now Loyola Marymount University) in 1953 with a B.S. in biology and chemistry. He had served in the U.S. Army from 1950 to 1952 during the Korean War, with service as a combat infantryman in North Korea. Holland subsequently completed his Ph.D. in microbiology at UCLA. For his doctoral research, he worked with M. John Pickett on genetics and immunity of *Brucella* infections in primary cells. From 1957 to 1959, Holland was a postdoctoral fellow at the University of Minnesota with Jerome Syverton, a virologist and chairman of the Department of Bacteriology. Holland wanted to do postdoctoral research with Syverton because of his expertise in primary and immortalized human and monkey cell cultures and his pioneering studies demonstrating the replication of different viruses in such cell lines. In fact, Holland had two postdoctoral mentors at Minnesota; Syverton had Holland work in Lee McLaren's lab. McLaren was an Assistant and then Associate Professor while Holland was there. Holland published his first virology papers while at Minnesota, providing fundamental knowledge about poliovirus replication. His work was the first to demonstrate that the block to poliovirus growth in nonprimate cells was due to the absence of specific receptors and that poliovirus nucleic acid alone could be used to infect nonsusceptible cells (1, 2). Beyond their crucial biological implications, these studies demonstrated how Holland would emerge as a central figure in applying molecular biology approaches to the study of viruses.

Holland was an Assistant Professor in the Department of Bacteriology and Immunology at the University of Minnesota from 1959 to 1961, and then he moved to the University of Washington, where he was an Assistant and then Associate Professor of Microbiology from 1961 to 1964. His research during this time focused on receptor affinities as major determinants of the tissue tropisms of enteroviruses (3). Holland and his colleagues also obtained some of the first convincing evidence that poliovirus replication occurred in the cytoplasm of infected cells and that poliovirus RNA could be encapsidated in coxsackievirus virions, providing the first demonstration of "enclosure of an animal virus genome

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within the capsid of a heterologous virus" by a nonenveloped virus (4, 5).

In 1963, Holland was awarded the American Society for Microbiology's Eli Lilly Award. The award is given annually for outstanding research of unusual merit, and at the time it was given to a scientist under 35 years of age. The award cited Holland's "research on the early stages of cell-virus interaction that culminated in a new concept of the dynamics of cell-virus interaction, new information on receptor development by cells, and an extensive documentation of the controlling role played by these receptors in infection." As an award recipient, he was invited to contribute a review article, which encapsulates his findings on the species, tissue, and cell specificity of poliovirus (6).

Holland was recruited to be the founding chairman of the Department of Molecular and Cell Biology (now called the Department of Molecular Biology and Biochemistry) at the University of California, Irvine (UC Irvine), in 1964, a year before classes started on campus. His first temporary lab at UC Irvine overlooked a majestic freshwater marsh adjacent to the campus (now known as the San Joaquin Marsh Reserve). Holland often remarked that this laboratory setting, although temporary and quite rustic, was one of the favorites he experienced in science. At Irvine, he embarked on experiments examining the fundamental molecular biology of mammalian cell protein synthesis, using virus-infected cells as a tool. He demonstrated that the host cell has little influence on the relative proportion of viral proteins synthesized but a profound role in determining the rate and total amount of viral protein and virion synthesis (7). In a seminal paper published in *Proceedings of the National Academy of Sciences* in 1968, Holland and his colleague Don Kiehn demonstrated that mRNAs of enteroviruses and other picornaviruses are translated to produce very large proteins that are then specifically cleaved into smaller functional proteins required for virus replication (8). This work, along with similar findings made independently by Don Summers and Jake Maizel and by Michael Jacobson and David Baltimore, had major implications for our understanding of how positive-strand RNA viruses generate their functional polypeptides, and it paved the way for the discovery of virus-encoded proteinases. Holland's work on picornavirus protein processing and replication was highly innovative and characterized by a "simple but elegant" experimental approach. These characteristics of Holland's approach to experimental virology formed a theme that ran throughout his entire research career.

In 1968, Holland was recruited to the Department of Biology at the University of California, San Diego. He began working on enveloped viruses and the composition of mammalian cell membranes at about that time. Most of his research was carried out with vesicular stomatitis virus (VSV), an enveloped, negative-strand RNA virus, but he also studied influenza and rabies viruses. At that time, a number of investigators were characterizing heterologous virus interference in coinfections. Holland and his laboratory colleague Mike Doyle demonstrated the dominance of poliovirus in a coinfection with VSV; VSV virion production is inhibited at the level of viral mRNA translation (9). Holland and colleagues also investigated homologous interference conferred by defective interfering particles of VSV *in vitro* and *in vivo*. Highly purified defective interfering particles in high doses protect mice against challenges of otherwise-lethal low doses of standard VSV and alter the disease process (10). This work led to subsequent findings that VSV defective interfering particles mediate persistent viral infections, in part by suppressing virion transcriptase (11). To further investigate persistence, Holland started long-term persistent infections of VSV with standard virus plus defective interfering particles. In the days before rapid RNA genome sequencing methods, Holland and colleagues documented VSV genome mutations by oligonucleotide fingerprinting in viruses derived from the persistent infections over a period of more than 5 years (12). This led to what are undoubtedly some of Holland's best known contributions among current virologists, in the area of RNA virus evolution. In a landmark treatise entitled "Rapid evolution of RNA genomes," Holland carefully reviewed the literature on RNA virus mutation frequencies, accumulation of RNA genome mutations, and RNA virus recombination and combined it with his knowledge of rapid evolution of RNA viruses during serial high-multiplicity passage and effects of defective interfering particles (13). He discussed the implication of high RNA virus mutation rates for persistent virus infections and human degenerative diseases and the implications for general evolution. According to Google Scholar, this paper has been cited nearly 1,200 times. Subsequent studies, many of which were carried out with his long-term collaborator Esteban Domingo, led to the now widely accepted concept of virus quasispecies (14). Using novel, quantitative experimental approaches, Holland and his coworkers greatly advanced our understanding of the molecular mechanisms that have generated genetic diversity among RNA viruses and, ultimately, among living organisms in general.

Holland served the virology community as a member of NIH, American Cancer Society, and U.S. Army grant review panels and journal editorial boards and as a coorganizer of an international colloquium on rhabdoviruses. He served on the Panel of Virus Diseases of the U.S.-Japan Panel for Cooperative Medical Sciences and the U.S. Institute of Medicine/National Academy of Sciences Committee on Microbial Threats to Health. Holland was also an excellent classroom teacher; his undergraduate medical microbiology course was consistently well received.

John's scientific legacy includes not only his more than 150 original research papers and review articles in respected and prestigious scientific journals but also his contribution to the careers of many others. He trained a significant number of Ph.D. students, postdoctoral fellows, and undergraduate researchers who now have academic positions in major universities and research institutions in the United States and Europe or positions in the biotechnology or pharmaceutical industries. Many of these individuals still carry out research in virology. Moreover, he graciously hosted visiting professors on sabbatical who were enriched by and themselves enriched the Holland lab. John's laboratory was unique: he worked in the lab with an uninhibited joy until retirement. This was educational, inspirational, and entertaining for those of us fortunate to work with him. He was a great model of how to do excellent science, think critically, and persist in the pursuit of knowledge. He could laugh at his own mistakes and empathize with other lab members' technical failures. After his trainees left the lab, he happily corresponded regularly with them about politics, sports, science, and philosophy until a few days before his death.

John had a rich life outside the laboratory, both before and after retirement. He enjoyed trout fishing and backpacking in the Sierra Nevada Mountains, which he began at an early age with his brother Rich. John continued the backpacking tradition with his son Mark, who recalls that John was his most relaxed and at home in the mountains. John's love of nature was also fulfilled by hiking in the desert in New Mexico and Nevada. He took pride in baking excellent French bread that he

shared with the lab, and in his typical thorough style, John built an outdoor wood-fired oven to produce the bread he had come to love while on sabbatical in Geneva, Switzerland. John was an avid vegetable gardener, and he also painted large oil landscapes. He had a single-engine private pilot's license and a glider pilot license and generously treated members of the lab to glider flights. In retirement, John enjoyed having more time to satisfy his intellectual curiosity. He researched specific topics related to quantum mechanics and also pursued an interest in powering internal combustion engines with carbohydrates, for which he received a patent in 2007 (15).

John is survived by his wife, Dottie (Mesquite, NV), his children, Mark (Albuquerque, NM) and Lynn, and grandchildren, Molly and Megan (San Clemente, CA), and three siblings, Mary Frieze (Los Angeles, CA), George Holland (Mammoth Lakes, CA), and Bob Holland (Mesquite, NV). John's beloved dogs, Milo and Millie, also survive him. Dogs were a very large part of John's life from childhood on, and one anecdote vividly illustrates that. When John returned from his army service in Korea, his dog had a moment of hesitation; then, when she remembered who he was, both the dog and John had the most joyous reunion, happy that they hadn't forgotten each other.

John once wrote that all three of his mentors, Drs. Pickett, Syverton, and McLaren, loved bench science, worked at the bench whenever they could all their lives, and were smarter and worked harder than John, and that he tried to emulate them as human beings. Not surprisingly, those of us who worked with John would say the same about him. He was also a model of scientific altruism, ethical behavior, and the highest standards of excellence. He had a profound impact on virology and scientists, and his intellect, wit, and imagination will be long remembered. An annual lectureship honoring John Holland at the American Society for Virology meeting has recently been announced.

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