Renato Dulbecco

1914–2012

BIOGRAPHICAL *Memoirs*

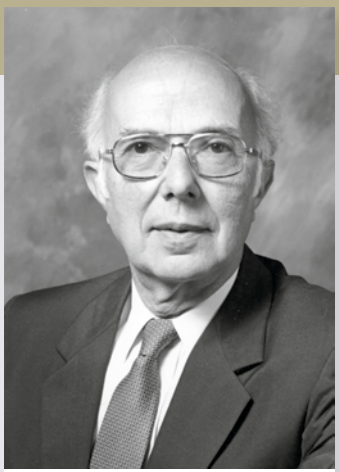
A Biographical Memoir by
David Baltimore

NATIONAL ACADEMY OF SCIENCES

RENATO **DULBECCO**

February 22, 1914–February 19, 2012

Elected to the NAS, 1961



By David Baltimore

Renato Dulbecco was a pioneering molecular biologist, virologist, and cancer researcher. He was born to Leonardo and Maria Dulbecco in Catanzaro, Italy, on February 22, 1914, and he died in La Jolla, California, on February 19, 2012. He was married twice, first to Giuseppina Salvo and later to Maureen Rutherford Muir. He is survived by Maureen, two children, and four grandchildren.

I got to know him when he invited me to set up my first laboratory within his space at the then-nascent Salk Institute for Biological Studies. Starting in 1965, I worked there for three years. He had come to the Salk Institute from a professorship at the California Institute of Technology (Caltech), where he had already established a notable career in virology.

Renato grew up in Porto Maurizio, Italy, a town of Liguria renamed by Mussolini as Imperia. It was near Turin, where he went to college.

He was a student in the memorable pre-World War II laboratory of Giuseppi Levi along with two other Italian students—Rita Levi-Montalcini and Salvador Luria—who, like him, eventually came to America and later won Nobel Prizes.

Renato became a physician and was conscripted into the Italian army to serve on the Russian front. While on the train through German-occupied territory, he saw a prisoner work gang. Not recognizing the special tattoo on each prisoner, he was horrified to learn later that as Jews they would be exterminated as soon as the construction was finished. He vowed to dissociate himself from such regimes as soon as he could.

At the front, after convalescing from wounds, he was sent back to Italy and continued his work as a physician and researcher. He attempted to establish his research career in Turin

after the war, but found that endeavor difficult. He studied both physics and mathematics with the belief that these were disciplines that would be important for a budding biologist. In this he was certainly prescient.

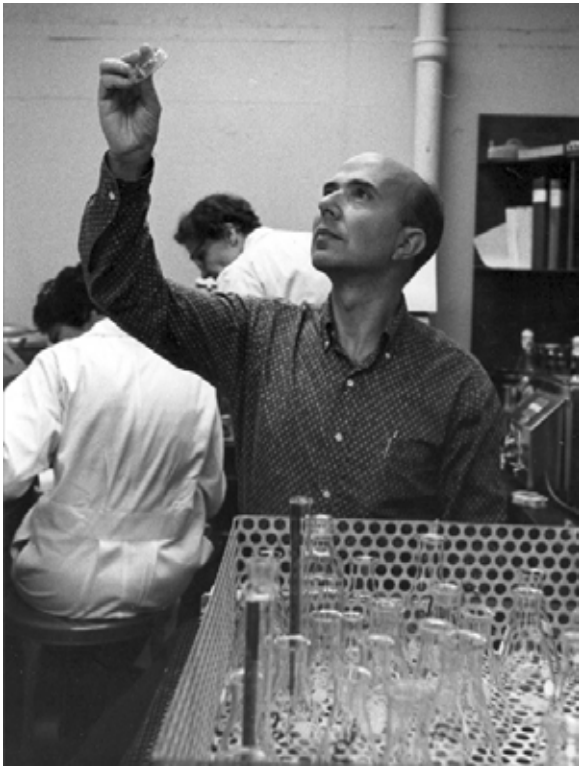
Meanwhile, Luria, at the start of World War II, had gone to France and later to the United States while Levi-Montalcini remained in Italy to study embryology. In the United States, Luria became a founding member of the "phage group," geneticists who used bacterial viruses (bacteriophages or just "phages") to study the fundamental processes of life. After the war, he occasionally visited Italy to see family, and on one trip he looked in at the laboratory of Giuseppi Levi, where he saw Renato again. He asked Renato to join him in Bloomington as a research associate at Indiana University, where Luria was a member of the faculty. Levi-Montalcini and Renato came to the United States on the same boat and then parted ways, one going to St. Louis, the other to Bloomington.

Thus, it was not until 1947 that Renato could begin his great career in virology. His story is similar to that of some other European scientists—Levi-Montalcini, Francois Jacob, and Jacques Monod, for example—whose scientific life was put on hold by the war and who could only develop their careers afterward. Each of them did work that greatly deepened our understanding of biology and each of them, including Luria, received Nobel Prizes.

In Luria's laboratory, Renato worked on aspects of bacteriophage genetics, notably discovering the process of photoreactivation of ultraviolet light (UV)-irradiated phage. It was an important and unexpected observation, showing that visible light energy could reverse the deleterious effects of UV irradiation. He published the work in a single-authored paper in *Nature* in 1949.

Renato spent a summer at Cold Spring Harbor meeting luminaries of the then-nascent field of molecular biology and coming to the attention of Max Delbrück. Delbrück—a pre World War II refugee from Germany, who was trained as a physicist—was a member of the faculty at Caltech and, on the basis of Renato's phage work, asked Renato to join him there. James Watson worked in Luria's laboratory at the time and advised Renato to go to Caltech, saying that Caltech was the best school of biology in the world and that he must accept the invitation. Only two years after arriving in the United States, Renato moved to Caltech and expected to continue his phage work.

A while after Renato's arrival at Caltech, a donor offered Delbrück $100,000 to establish work on animal viruses. Delbrück suggested that someone in his phage group take this as an opportunity to switch into animal virus research. Renato accepted this challenge because he recognized it as a wide-open field and was attracted to the idea of working on viruses of medical importance. Although only a senior research fellow at Caltech, Renato set out to solve what he saw as the key problem holding back animal virology: the lack of a quantitative assay to identify live virus particles. Without such an assay, it was hard to do quantitative experimentation. The whole history of phage work had shown the centrality of this issue through the use of a plaque assay.



Renato Dulbecco in Caltech lab. December 1961.
(Photo by James McClanahan.)

Live phage could be counted by diluting a preparation to about ten to one hundred particles per milliliter and spreading one milliliter on an agar surface, along with a concentrated culture of bacteria. The live bacteria would grow, while those infected by the phage would explode (lyse) and release many phage progeny that could infect the bacteria in the neighborhood. In the end, a lawn of bacteria would form with holes where a live phage in the original preparation had landed. The number of holes showed the original number of live phage.

Renato reasoned that to assay an animal virus, a monolayer of mammalian or chicken cells growing on the surface of a glass Petri dish could take the place of the bacterial lawn. A dilute agar gel containing the nutrients for the cells could be placed over the top. Agar, rather than liquid medium, would keep the released virus from spreading over the plate by convection. And, by carefully adjusting the light, plaques of dead cells could almost be seen.

Renato later realized that he needed a dye to provide better contrast between the lysed cells and live ones. This he found in a chemical known as neutral red, which is concentrated by live cells but excluded from dead cells. With that, anyone could see the plaques, and quantitative animal virology was born.

Renato's first paper on a plaque assay for western equine encephalitis virus using chicken embryo fibroblast cells, a single-authored paper in 1952, set the stage for all further quantitative analysis of animal viruses.

From left: Rita Levi-Montalcini, Renato Dulbecco, Melvin Cohn, Edwin Lennox, and Marguerite Vogt at Torrey Pines Mesa in 1964. (Photo courtesy the Salk Institute.)

In the early 1950s, the most-feared virus in America was poliovirus, the one that caused poliomyelitis, the disease that left Franklin D. Roosevelt paralyzed. At the urging of the National Foundation for Infantile Paralysis, Renato turned his attention to this virus, hiring an associate to concentrate on the problem. She was Dr. Marguerite Vogt, the daughter of a famous German neurologist, who came to Delbrück as a refugee looking to make a scientific career in America. Vogt was a wonderful woman who became Renato's life-long scientific associate until her death in 2007. She was an intense scientist with a huge heart who is remembered fondly by all who were ever in Renato's laboratory.

My wife Alice S. Huang and I worked for many years on quantitative aspects of virus growth and viral genetics, some of it on poliovirus. We owe a huge scientific debt to Renato and Vogt for their painstaking work, which made animal virology an easy field to break into and helped us to establish our careers.

In 1953, Renato decided to expand his horizons and accepted as a post-doctoral researcher Harry Rubin, a veterinarian who wanted to study viruses that cause cancer. Up until then, Renato had been focusing on viruses that kill cells, but Rubin brought a new

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concept to the laboratory: that a virus could grow in a cell but not kill it and could so profoundly affect the metabolism of the infected cell that it would take on the properties of a cancer. This was known from the work of others, but just as Renato had realized earlier in relation to viruses that killed cells, if cancer were to be understood through viruses, a quantitative assay was needed.

Rubin worked on many aspects of the cancer-inducing avian virus Rous sarcoma virus (RSV) until 1957, when a Caltech graduate student, Howard Temin, joined them. Temin set about creating the quantitative assay for RSV and his success was recorded in a 1958 paper by Temin and Rubin in Virology. (Characteristic of Renato, this seminal paper from his laboratory, as well as all of the many Rubin papers from Caltech, does not include his name as an author.)

Temin went on to develop the hypothesis that cancer-inducing viruses could be carried by normal cells, and in this form he referred to them as "proviruses." But RSV has RNA as its genetic material, while a provirus would require DNA. This led to the theory that DNA and RNA might be interconvertable, an idea that Temin and I pursued independently, searching for a reverse transcriptase.

Rubin's presence in Dulbecco's laboratory had a profound effect on Renato. He realized that viruses might be used to probe for the genetic origins of cancer. In 1958, the role of genes in cancer was still a very debatable subject. But in that year, Sarah Stewart and Bernice Eddy at the National Cancer Institute isolated the mouse polyoma virus, a small, DNA-containing virus that grew in cell cultures and caused cancer when inoculated into rodents. Renato quickly switched his laboratory activities to focus on this virus and a close simian relative, SV40 virus. He rightly saw that with only a limited amount of genetic material, these viruses might hold the key to understanding how genes could cause cancer.

First, the Dulbecco lab had to tame the virus and learn how to deal with it in the laboratory. They showed that it could be assayed on one cell line by plaque formation, and that in another cell line it caused cancerous foci (a phenomenon called transformation) but it did not reproduce. They termed these "permissive" and "non-permissive" cell lines,

respectively. They isolated significant quantities of virus, extracted the DNA, and showed that it was a small, circular molecule. This had important implications for the duplication of the DNA and for its possible integration with the host cell chromosome.

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Dulbecco receiving the Nobel Prize from the King of Sweden.

Work on this question was undertaken by Joe Sambrook, a post-doctoral fellow, and others in Renato's laboratory. They showed in 1968 that viral DNA was present in the transformed cells and that it was covalently attached to the cellular chromosome. In other words, transformation involved integration of a viral chromosome with the host cell's chromosome. Thus, it became possible that viral genes were expressed in a transformed cell and that the original virus contained cancer-causing genes. The integration of viral genes also explained why transformed cells remain cancerous and do not revert to normalcy.

The next step in their research was to show that the viral genome in the transformed cell encoded virus-specified messenger RNA molecules. Members of Renato's laboratory reported this finding in 1968, but thereafter the research became very difficult because it was unclear which protein caused the transformation. Multiple laboratories working over decades have finally resolved many of these issues, showing that transformation is actually induced by three proteins working through an elaborate process, but the explanation needn't detain us here.

Members of Renato's laboratory went on to study many aspects of cell transformation. One of their most significant observations was that the infection of quiescent cells with polyoma virus induced cellular DNA synthesis. This meant that the virus was able to subvert cellular controls in normal cells that inhibit DNA synthesis and prevent growth.

Virus-transformed cells do not stop growing, even when nutrients are limited. This is why they are considered cancer cells.

The Nobel committee chose to honor Renato, Howard Temin, and me with the Nobel Prize in Physiology or Medicine in 1975 for "discoveries concerning the interaction between tumour viruses and the genetic material of the cell." Renato's work had set the stage for understanding how DNA tumor viruses transform cells; the work Howard and I did set the stage for understanding how RNA tumor viruses transform cells. It is remarkable that all three of us were so tightly associated and yet never published our research together. In spite of that, both Temin and I were proud to say that we spent time with Renato and that he claimed us as his students in the prologue to his autobiography. We were particularly influenced by his clarity of thought on very difficult problems. However, many others who worked with him and did publish with him went on to lustrous careers as well, notably two other Nobel laureates, Lee Hartwell and Susumu Tonegawa.

Following the awarding of the Nobel Prize, Renato chose to examine more biological aspects of cancer. He was especially intrigued by breast cancer, studying it mainly in rats. He continued this interest until his death, publishing as late as 2008 (when he was ninety-four years old) with a group that he maintained in Milan, Italy, in his later years. But in the years after 1975, Renato came to the realization that cancer is a multi-faceted problem that will take decades to unravel.

In particular, he recognized that much of the complexity of cancer derives from the multitude of genes and related gene products that interact to regulate the growth cycle of a cell, interactions that must be countered when cells become cancerous. However, he also knew that we were yet to understand the full range of genes that act in cells. He saw over the horizon the possibility of sequencing the whole human genome and thus providing cancer research with a sorely needed catalog. In 1986 he wrote a two-page perspective in Science magazine entitled, "A Turning Point in Cancer Research: Sequencing the Human Genome." In it, he focused on the sequential mutations that progressively drive a cell clone to become a malignant cancer. He noted that this sequence could imply a daunting genetic complexity and said providentially, "We have two options, either to try to discover the genes important in malignancy by a piecemeal approach, or to sequence the whole genome of a selected animal species." That species, he said, should be humans. He strongly argued for whole-genome sequencing, recognizing that it would have utility far greater than being a window on the cancer problem.

Maureen and Renato Dulbecco.

However, he saw that it was a task too great for any one laboratory, and so he called for an "international undertaking." It took many years of effort to bring his dream to reality, but there is no question that he initiated the process.

Renato was a gracious man who was quite formal in his dress and demeanor. He loved music, played the piano well, and he was a widely read intellectual. He cared deeply about the welfare and products of science, but when his laboratory trainees gathered at his house on social occasions, he insisted that they discuss subjects other than science. This often resulted in long silences. During one such occasion, he proffered the opinion that the Beatles were a singular phenomenon. I wish now that we had asked him for a more detailed discourse on the subject, especially after hearing him play the piano once by accident.

That music was important to him is evidenced by a 2005 addendum to his biography on the Nobel Prize website. He had been traveling often to Italy, where he had scientific

groups, but said in the addendum, "At the beginning of 2006, when I will reach 92 years of age, I will give up the Italian connections, and will retire at La Jolla, to follow the work going on at the Salk Institute, and to play the piano."

As I knew him, in his later years, he was a deeply involved family man helping to raise a wonderful daughter, Fiona, who became a well-known cardiologist in San Francisco. To his last days, he took great pride in her successes. Much credit must go to his wife Maureen, who was perhaps more effectively grounded in day-to-day reality and often seemed to guide Renato through it. Although it was not obviously in his nature to take on a bureaucratic position, he was president of the Salk Institute more than five years, instantiating his love for the institute, an affection that is shared by so many of us who have spent time at the Salk Institute. He was one of the institute's creators, present at the start, when Jonas Salk and Leo Szilard selected a faculty to set the direction of the institute, which has remained firm to this day.

Renato lived through the revolution that we now call molecular biology and he led its application to animal viruses, mammalian cell biology, and cancer. Interestingly, another person who led that revolution was his mentor, Luria, and I count both of them as my guides. There are few people left who participated in the birth of molecular biology, but the imprint of this band of innovators on our understanding of the nature of life is indelible. Renato was one who played multiple roles, every time seeing over the horizon to what was possible and finding ways to bring us to that new level of understanding.

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REFERENCES

Nobel Media AB (2013). Renato Dulbecco – Biographical. Available online at http://www.nobelprize.org/nobel_prizes/medicine/laureates/1975/dulbecco-bio.html.

Salk Institute for Biological Studies (2012). "Renato Dulbecco, Nobel laureate and pioneering cancer researcher, dies at 97." Available online at http://www.salk.edu/news/pressrelease_details.php?press_id=545.

Daniel Kevles (1993). Renato Dulbecco and the New Animal Virology: Medicine, Methods, and Molecules. *Journal of the History of Biology* 26:409–442. Available online at http://www.jstor.org/stable/4331279.

Shirley K. Cohen (2001). Interview with Renato Dulbecco, Pasadena, California, September 9 and 10, 1998. *Oral History Project*, California Institute of Technology Archives. Available online at http://resolver.caltech.edu/CaltechOH:OH_Dulbecco_R.

Renato Dulbecco recorded a series of video interviews that are available through Web of Stories, an online archive project run by the London-based Science Navigation group, at http://www.webofstories.com/story/search?q=dulbecco. The interviews are conducted in Italian and subtitled in English.

SELECTED BIBLIOGRAPHY

1949 Reactivation of ultra-violet-inactivated bacteriophage by visible light. *Nature* 163: 949.

The number of particles of bacteriophage T2 that can participate in intracellular growth. *Genetics* 34:126–32.

With S. E. Luria. Genetic recombinations leading to production of active bacteriophage from ultraviolet inactivated bacteriophage particles. *Genetics* 34:93–125.

1952 Production of plaques in monolayer tissue cultures by single particles of an animal virus. *Proc. Natl. Acad. Sci. U.S.A.* 38:747–52.

1953 With M. Vogt. Some problems of animal virology as studied by the plaque technique. *Cold Spring Harb. Symp. Quant. Biol.* 18:273–279.

1954 With M. Vogt. Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. Exp. Med.* 99:167–182.

With M. Vogt. One-step growth curve of Western equine encephalomyelitis virus on chicken embryo cells grown in vitro and analysis of virus yields from single cells. *J. Exp. Med.* 99:183–199.

1956 With M. Vogt and A. G. Strickland. A study of the basic aspects of neutralization of two animal viruses, Western equine encephalitis virus and poliomyelitis virus. *Virology* 2:162–205.

With G. Freeman. Plaque production by the polyoma virus. *Virology* 8:396–397.

1960 With M. Vogt. Significance of continued virus production in tissue cultures rendered neoplastic by polyoma virus. *Proc. Natl. Acad. Sci. U.S.A.* 46:1617–1623.

With M. Vogt. Virus-cell interaction with a tumor-producing virus. *Proc. Natl. Acad. Sci. U.S.A.* 46:365–370.

1962 With M. Vogt. Properties of cells transformed by polyma virus. *Cold Spring Harb. Symp. Quant. Biol.* 27:367–374.

1963 With M. Vogt. Evidence for a ring structure of polyoma virus DNA. *Proc. Natl. Acad. Sci. U.S.A.* 50:236–243.

1965 With L. H. Hartwell and M. Vogt. Induction of cellular DNA synthesis by polyoma virus. *Proc. Natl. Acad. Sci. U.S.A.* 53:403–410.

1967 The induction of cancer by viruses. *Sci. Am.* 216:28–37.

1968 With J. Sambrook, H. Westphal, and P. R. Srinivasan. The integrated state of viral DNA in SV40-transformed cells. *Proc. Natl. Acad. Sci. U.S.A.* 60:1288–1295.

1969 With M. Stoker. Abortive transformation by the Tsa mutant of polyoma virus. *Nature* 223:397–398.

1975 Oncogenic viruses: the last twelve years. *Cold Spring Harb. Symp. Quant. Biol.* 39:1–7.

1986 A turning point in cancer research: sequencing the human genome. *Science* 231:1055–1056.

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