Giuseppe Attardi

1923–2008

BIOGRAPHICAL *Memoirs*

A Biographical Memoir by
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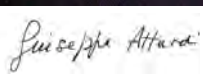
GIUSEPPE **ATTARDI**

September 14, 1923–April 5, 2008

Elected to the NAS, 1984

Photography by Jim Staub, California Institute of Technology



By Anne Chomyn

Giuseppe Attardi, an Italian American scientist, obtained a degree in medicine from the University of Padua, but he never intended to practice medicine. He left his native land several years later to become a part of the emerging field of molecular biology, where he made significant contributions in the field of gene expression in bacterial, avian, and mammalian cells.

Giuseppe is best known for his in-depth characterization of mitochondrial RNA and its synthesis in mammalian cells. His discovery that more than half the informational content of mitochondrial DNA (mtDNA) is dedicated to coding for subunits of complex I, the largest enzyme of the respiratory chain, completed the elucidation of the mitochondrial genome. In addition, his laboratory produced the first mammalian cell lines that lack mtDNA. These cell lines have become important genetic tools in the study of mitochondrial diseases.

Early years

Giuseppe was born in Vicari, Sicily, in 1923. He was the second of three sons born to Luigi Attardi, a Sicilian, and Saveria, a Calabrian. Giuseppe's father was a prefect, a representative of the Ministry of the Interior. While the children were growing up, the father's position required them to relocate to several cities, namely L'Aquila, Benevento, Pula (now a part of Croatia), and, finally, to Padua in 1939. This last move was fortunate, as the city had an outstanding university that all three sons eventually attended. Giuseppe's parents and brothers lived in Padua for the rest of their lives. His brothers Stefano and Aldo studied law at the university, the former eventually becoming a military judge, and the latter, a professor of law.

Giuseppe entered the University in 1941. Italy had entered the war on June 10, 1940, while France was being defeated by Germany. Giuseppe liked to recount how he managed to avoid serving in the Fascist army. His sympathies were with the partisans rather than with the Fascists. Moreover he did not want to interrupt his studies. When

he was called up by the army for his pre-induction physical exam, he prepared for it by sleeping only a few hours the night before, bicycling to the exam at top speed and underdressed for the winter weather, and then smoking the only three cigarettes he ever smoked in his life. To his great relief, he failed the exam.

Padua was bombed several times between September 1943 and April 1945. Studying medicine during that time was a test of Giuseppe's fortitude. He worked behind shuttered windows and sometimes with the sound of American bombs exploding in the distance. Occasionally sirens would sound and, at his parents' request, he and his brothers would bicycle out of the city and wait out the bombing from the safer outskirts.

Giuseppe completed his medical studies in 1947. However, he did not become a physician as his father wished. He had known from the beginning of medical school that he would pursue a career in research and not practice medicine. In fact, at that time in Italy, the way to become a biologist was to get a medical degree. Giuseppe had already published two papers while he was a student. One was on the structural characteristics of adrenal gland veins. This work was done in the laboratory of his professor, Luigi Bucciante, who used tissue culture to study anatomical systems and had a particular interest in blood flow. In the second paper, Giuseppe and fellow medical student Luigi Marcon, deviating from the interests of their professor, reported their observations on the antibiotic properties of *Penicillium* and of *Aspergillus*, using bacteria on agar plates as their test system.

The publication of this second paper fueled Giuseppe's enthusiasm and ambition and led to an exciting but, in the end, sobering venture. Giuseppe and Marcon wanted to continue their work with antibiotic molds, perhaps with the idea of developing therapeutic applications there in Italy. They hoped to get a certain industrialist, located in a distant town, interested in supporting further work. The evening before their appointment with him, they took a train to the town, arriving with very little money in their pockets. They had to choose between dinner and a room in a hotel. They chose the dinner and slept under a bridge.

The next day the industrialist listened to their proposal and agreed to provide support to carry out pilot experiments in animals. After some time passed, Giuseppe and Marcon had to show their sponsor the results of their work. They had made a pigeon sick with an infection and treated it with one of the antibiotic-producing molds. When they tossed the pigeon into the air, it barely managed to flap its wings and then it dropped to the ground, bringing their high hopes down with it.

The Portal "Heart"

Shortly after receiving his medical degree, Giuseppe was made assistant professor of histology and general embryology at the University of Padua, a position he kept until 1957. In Italy an assistant professor was attached to a professor, so Giuseppe continued to work with Bucciante. Giuseppe excised segments of arteries from chick embryos and put them into culture in rich medium. He observed that in about ten percent of his cultures, the arteries pulsated autonomously and could continue to do so for as long as three days. The first paper on this subject was co-authored by Enrico Gandini and Luigi Marcon, and was published in an Italian journal (1948).*

A year later, Giuseppe married Gandini's sister, Domenica (Nica) Gandini. Nica had also been a medical student at the University of Padua. She began working with Giuseppe and published two papers with him before her research interests took a different path. Their son, Luigi, was born in 1952. Luigi became a poet, filmmaker, and translator. He wrote under the penname Nail Chiodo. He died in 2014.

Giuseppe's work on blood vessels culminated in the discovery of what he called the "portal heart…that marvel of physiological hydrodynamics that is represented by the portal vein of rodents" (1955). He found that the portal vein exhibited a strong peristaltic contractile activity that persisted *in vitro* for as long as fifteen days. He observed *in situ* that the portal vein is lined with spiral folds. In the relaxation phase these folds serve to mix the blood coming in from the spleen and the intestines. Then, as the vein contracts, the folds are absorbed into the walls of the vein and blood is pushed forward into the liver. Thus, this "portal heart" alternately mixes and pushes the blood. In later years Giuseppe often recalled these experiments and the beauty of the "portal heart" with a great deal of nostalgia.

Torbjörn Caspersson and the Karolinska Institutet

Giuseppe's interests then turned to nucleic acids. There were very few laboratories in the early 1950s working on DNA and RNA. One of the two leading laboratories in the field of RNA was that of Torbjörn Caspersson at the Karolinska Institutet in Stockholm. Giuseppe arranged to work with Caspersson in 1952 and returned several times over the next few years.

* Items noted with a year, e.g. (1948) can be found in the selected bibliography. Items annotated with a number, e.g. (1), can be found in the references.

Rita Levi-Montalcini and Giuseppe Attardi at ceremony following the award of the Antonio Feltrinelli Prize for International Medicine to Giuseppe in 1989. (Photograph courtesy of Accademia Nazionale dei Lincei.)

Caspersson had proposed that there was a relationship between RNA and protein, and that cells containing high levels of RNA were synthesizing or were set to synthesize increased amounts of protein. He built an ultramicrospectrophotometer that scanned tissue sections on slides and quantified nucleic acids (comprising mostly RNA) in cells of the tissue (1). Giuseppe carried out an investigation to determine whether the RNA levels in Purkinje cells of the rat cerebellum could be measured with the new instrument, and if so, whether these RNA levels would be affected by stress from constant motion. In this work, Giuseppe combined his interest in RNA with his interest in the nervous system, which had developed during medical school. He published two papers from this period, one demonstrating that the RNA levels in Purkinje cells were quite variable from cell to cell, but that the mean was reproducible (1953). The second paper demonstrated that after the rats were subjected to constant motion, the RNA levels of the Purkinje cells were significantly elevated (1957).

Rita Levi-Montalcini

In 1955, at an international conference on anatomy in Paris, Giuseppe presented a paper on the autonomous peristaltic activity of the portal vein and the behavior of the spiral folds. At this conference he met Rita Levi-Montalcini for the first time. She had a position then at Washington University in St. Louis, Missouri. Levi-Montalcini had received an MD from the University of Turin and had studied with Giuseppe Levi, who had been the mentor also of Salvatore Luria, Renato Dulbecco, and Giuseppe's professor, Luigi Bucciante. She was to win, along with Stanley Cohen, the Nobel Prize in Physiology or Medicine in 1986 for their discovery of Nerve Growth Factor.

Levi-Montalcini became a lifelong friend, mentor, and supporter of Giuseppe. She advised him then, when it was still early in his career, to go abroad to learn modern

biology. First, however, he would have to take courses in physics and mathematics, because molecular biology was an exquisitely quantitative science, because it often included the use of radioactivity, and because several leaders in the nascent field had a background in physics. Giuseppe enrolled himself in a two-year program in physics, which he completed in one year. Giuseppe's decision to follow Levi-Montalcini's advice was a crucial turning point in his career.

Melvin Cohn and Washington University

Levi-Montalcini arranged for Giuseppe to work with her friend and colleague, Melvin "Mel" Cohn, in the Department of Microbiology at Washington University. Giuseppe went to Cohn's laboratory with a Fulbright Fellowship, taking a two-year leave from his position as assistant professor in Italy to become a postdoctoral fellow in the United States. Cohn, originally an immunologist, had worked at the Pasteur Institute with Jacques Monod on the induction of enzyme production in bacteria. At Cohn's suggestion, Giuseppe went to Paris first, in the fall of 1957, to take a two-month course on the emerging field of molecular biology that was taught by Monod, François Jacob, and others of the Pasteur Institute.

Giuseppe arrived at Washington University near the end of 1957. The Department of Microbiology at that time was headed by Arthur Kornberg. Other members of the Department included Paul Berg, Dale Kaiser, David Hogness, and Robert Lehman. The Department provided a rich and congenial environment for learning about modern biology. There were departmental journal clubs and an unorthodox textbook-less microbiology course taught by each of the aforementioned department members, including Cohn.[i]

Cohn's interests at that time were returning to immunology. The central question in the field was whether an antibody-producing cell had the capability of producing multiple antibodies (the instructionist mechanism), or whether an antibody-producing cell was pre-programmed to produce antibodies of defined specificity. This latter idea was the basis of the "clonal selection" theory, which, though formulated earlier, gained widespread attention only in 1959 (2).

Cohn, Ed Lennox, and Kengo Horibata had devised a hanging-drop method to test the number of specificities produced by a single cell. They incubated single B cells in a hanging drop for several hours to allow the accumulation of secreted antibodies.

The antibodies could then be recovered from the drop and tested for their specificity. Giuseppe decided to work on this project.

Other laboratories at that time were finding that each B cell produces only a single antibody idiotype. Yet, during painstaking work in which single cells were isolated from lymph nodes of rabbits that had been hyper-immunized against T2 and T5 bacteriophage (the antibodies produced from such cells were tested for their phage-neutralizing activity), Giuseppe, Cohn, Horibata, and Lennox were surprised to discover[ii] that about fifteen percent of cells producing antibodies to T2 or T5 could produce antibodies recognizing both bacteriophages.

Their first paper was published in 1959 (1959). By then, the immunology community was starting to subscribe to the "clonal selection" theory, which predicted that antibody-producing cells made antibodies of only a single specificity. The community met these results with "aggressive disdain," according to Cohn, and for some years other immunologists "peripheralized" the Cohn group.[iii] This reception did not encourage Giuseppe to stay in immunology. Now, as virtually the entire Microbiology and Immunology Department was on the point of moving to Stanford University, Giuseppe thought it might be time to go to another laboratory so that he could gain experience in another area of research. With letters from Cohn and Levi-Montalcini, he arranged to join the laboratory of Renato Dulbecco at the California Institute of Technology (Caltech). Meanwhile, the Cohn group carried out a number of control experiments to counter the skepticism that they had met, publishing five papers in 1964 (1964a-e). In the end, their original results stood.[iv]

Renato Dulbecco and Caltech

Giuseppe, his wife, Nica, and their son, Luigi, left Missouri for California in the summer of 1959. Along the way they visited various sights in the Southwest, Nica driving the green 1955 Pontiac as Giuseppe recorded the scenery on 8mm film. This was an exciting trip for them, and it was a move that would have important consequences for Giuseppe's career.

At Caltech, Dulbecco had established a large laboratory for animal virology in the sub-basement of the Church building. He had adapted the principles of virus quantification that he had learned in the bacteriophage laboratory of Max Delbrück, and he had developed methods of animal virus propagation, maintenance, and quantification by plaque assays. Among the viruses under study in the laboratory were polyoma virus, poliovirus, and Rous sarcoma virus.

Giuseppe, having experience with proteins, joined forces with John Derek Smith, a chemist from the Medical Research Council in Cambridge, England, who was expert in chromatography of nucleosides and nucleotides, to investigate what happened inside cells after infection by poliovirus. At that time, Sydney Brenner and François Jacob were visiting Caltech to work with Matt Meselson, hoping to establish that there was an RNA intermediate between DNA and ribosomes, namely messenger RNA (mRNA). In fact, they did experiments just down the hall from the Dulbecco laboratory. Thus Giuseppe and John Smith became aware of this idea of mRNA. Giuseppe and Smith demonstrated that in poliovirus-infected cells, normal cellular proteins were no longer synthesized, and instead, the cell's ribosomes were synthesizing poliovirus proteins. Furthermore, they showed that RNA associated with ribosomes in polio virus-infected cells had the same base composition as the poliovirus RNA genome, suggesting that the RNA detected on ribosomes was of poliovirus origin. This work was published two years later (1962). The same paper presented evidence of polysomes containing poliovirus RNA, though polysomes were still unknown and unnamed at that time.

François Gros and Paris

Toward the end of 1960, after more than a year in Dulbecco's laboratory, Giuseppe had to leave the United States because his exchange-visitor's visa was about to expire. He had already received an offer of a faculty position at the Massachusetts Institute of Technology, which he had to turn down because he was required to spend two years in his home country or a partner country before returning to the United States. Cohn, having connections in Paris, arranged for Giuseppe to work with George Cohen, who had just been named Director of the Centre National de la Recherche Scientifique (CNRS) in Gif-sur-Yvette, a suburb of Paris. Giuseppe moved with his wife and son to Paris for the next two years. During that time, his marriage to Nica came to an end. It was an amicable separation, the two maintaining a cordial relationship afterward.

On his first visit to Gif-sur-Yvette, Giuseppe found that the laboratory was still under construction. It would be six to eight months before the building was completed. Not to waste time, Giuseppe began frequenting the laboratory of Jacques Monod at the Pasteur Institute in Paris to meet and talk with people and to participate in seminars. François Gros was there and had recently, with James Watson, Walter Gilbert, and others, presented evidence for the existence of mRNA^{v} (3). Learning of Giuseppe's work with poliovirus RNA, François Gros invited Giuseppe to work with him at the Pasteur.

Lab party, 1966, left to right: Francesco Amaldi, Giuseppe Attardi, Barbara Attardi, David Kabat, Sue Kabat, Carol Hatlen, Loren Hatlen, Paola Amaldi, Laverne Wenzel, Anne Huberman. (Photo courtesy Joel Huberman.)

mRNA had been identified as an unstable RNA that could be detected by pulse labeling with radioactive uridine or phosphorous and, under some conditions, it was associated with ribosomes. Working with François Gros, Giuseppe showed that pulse-labeled RNA isolated from *E. coli* (a species of bacteria) could hybridize (that is, form duplexes) with denatured, single-stranded *E. coli* DNA, but not with a heterologous DNA (1961). Giuseppe then showed, using two different genetic systems, that induction of specific genes to produce proteins stimulated the synthesis of RNA that was complementary to the expressed genes (1963). This RNA was presumably mRNA. His work established that in those bacterial systems, gene expression is regulated by transcription of the gene.

Caltech

While in Paris, Giuseppe was offered a faculty position at Caltech, from which Dulbecco had left in 1962 to join the Salk Institute. Giuseppe arrived at Caltech in 1963 and moved into Dulbecco's former space in the subbasement of the Church building. He was to remain at Caltech for the rest of his life.

A few months after arriving at Caltech, Giuseppe met Barbara Furman. She was a Cornell undergraduate and a summer student in Robert Sinsheimer's laboratory. A romance developed and they were married in 1964. After getting her bachelor's degree, Barbara returned to Caltech for graduate school. She worked with Giuseppe for several years, during which time she earned a PhD and made landmark contributions to Giuseppe's research program. Giuseppe and Barbara have a daughter, Laura Attardi, who is now a Professor at Stanford University in Radiation Oncology and Genetics.

Ribosomal RNA

While Giuseppe is most known for his work on mitochondrial DNA (mtDNA) transcription, he also made important contributions to the understanding of the nuclear-encoded ribosomal RNAs and their genes. In fact, the first two papers from Giuseppe's laboratory at Caltech described the recognition of ribosomal RNA (rRNA) sites in the DNA of *E. coli* and of HeLa cells (a human cell line). Giuseppe had done his first hybridization experiments in Paris. Now at Caltech, he exploited this technique and trained his collaborators to master it as well, sometimes in very technically challenging experiments.

Giuseppe, together with his first post-doctoral fellow, P. C. Huang, and research assistant Susan Kabat, determined by hybridization experiments how much of the DNA of the two cell types mentioned above was used to encode the rRNAs (1965a, b). In this way they estimated the number of copies of the rRNA genes in the respective genomes. Later, in another paper, the fractions of the nuclear DNA used to encode 5S and tRNA were also determined (1971).

Together with Francesco Amaldi, the first of many Italian postdoctoral fellows who worked in his lab, Giuseppe derived partial sequences of the rRNAs from HeLa cells and other human cells. They and Philippe Jeanteur discovered the precursors to HeLa cell rRNA, namely the 45S and 32S RNAs, and they obtained partial sequences of these, as well (1968b).

In 1974, before the days of cloning, Giuseppe and graduate student Bill Murphy purified single-stranded DNA that was complementary to 28S and 18S rRNA from a preparation of total cellular DNA (1974). They did this using techniques available to them at the time: namely, DNA denaturation, reannealing (or strand reassociation), DNA-RNA hybridization, and digestion with nucleases. Giuseppe named these two purified single-stranded DNAs "probes." These rDNA probes were then used in hybridizations with 18S and 28S rRNAs for length determination by electron microscopy, and they were used in hybridizations with the 45S precursor RNA for mapping the regions on the precursor that corresponded to the 18S and 28S rRNA sequences. This was, I believe, the first use of the term "probe," which came into common use later to describe DNA fragments, usually prepared from plasmid clones, that are used in hybridization experiments with DNA or RNA, in solution or on gel blots.

Heterogeneous nuclear RNA

While the work with rRNAs proceeded, Giuseppe was investigating the synthesis of non-ribosomal RNA in eukaryotic cells. He used metabolic labeling with radioactive precursors to study rapidly synthesized RNA in duck erythrocytes and in HeLa cells. What Giuseppe discovered was very large, heterogeneous, unstable RNA confined to the nucleus (1966a, b). RNA of this size had never been observed before in bacterial or animal cells, with the exception of viral RNA. This large RNA came to be called "heterogeneous (Hn) nuclear RNA" and was discovered simultaneously and independently by James Darnell at The Rockefeller University (4).

These very large HnRNA species, some of which migrated with a sedimentation coefficient of 80S, reflecting a size of 2 X 10^7 daltons (30,000 nucleotides long) or more, did not appear to be exported to the cytoplasm and were not related to rRNA. Many years later, in 1977, researchers discovered that HnRNAs are in fact precursors of mRNA. The HnRNA molecules contain introns (non-coding regions), often several and often constituting much more than half of the gene, that are excised in a very precise manner in a process that ultimately forms mature mRNA.

Mitochondrial RNA

Whereas HnRNA was intriguing, it seemed to be too complicated to study, and at that time it was not certain that it was related to mRNA. Giuseppe looked for a more tractable, non-viral genetic system in eukaryotic cells for studying mRNA synthesis. One attractive system at the time was the avian erythrocyte, which was specialized for synthesizing the protein globin, the precursor to hemoglobin. Giuseppe and Barbara started working on a project to purify globin mRNA by immunoprecipitating polysomes from duck erythrocytes with antibodies to hemoglobin. However, they soon switched their attention to mitochondria. Margaret Nass had recently shown that various cell types of seven animal phyla and at least some plants had DNA inside their mitochondria (5). Mitochondria are cytoplasmic membrane-bound organelles that carry out many functions, chief of which is the production of the energy-rich ATP molecule in conjunction with the oxidation of metabolites. Giuseppe thought that perhaps this DNA had genes that were expressed. Using HeLa cells, he and Barbara found that there was a class of cytoplasmic RNA, which was largely associated with membrane structures, that incorporated radioactive precursors very rapidly, with no lag in appearance of radiolabeled RNA (1967). RNA in free polysomes, on the other hand, became radiolabeled only after a short lag, presumably reflecting the time it took for RNA synthesized in the nucleus to

be processed and transported out of the nucleus. Also, the rapidly labeled membrane-associated RNA had a different base composition from that of free polysome-associated mRNA.

Barbara soon showed that the rapidly labeled RNA, a mixture of species of different sizes, was hybridizable to purified mitochondrial DNA (mtDNA) (1968a). MtDNA was known to be a double-stranded circular molecule present in many copies per cell (6). It could be separated from the bulk of nuclear DNA by cesium chloride-ethidium bromide density gradient centrifugation (7), a technique that had just been developed by Jerry Vinograd and collaborators down the hall in the Church building sub-basement.[vi] Thus, the rapidly labeled membrane-associated RNAs were transcripts of mtDNA. This was the first demonstration of transcription of mtDNA.

The discovery of mitochondrial RNA synthesis opened up a new avenue of research in Giuseppe's laboratory (1970). Within a few years, Giuseppe, together with postdoctoral fellow Yosef Aloni, demonstrated that both strands of mtDNA are transcribed, each along virtually its full length. Giuseppe and collaborators found that human mitochondria had ribosomes, as did mitochondria of other species, and the mitochondrial rRNAs were among the mitochondrial transcripts. These were 12S and 16S in size, smaller than any other known rRNAs. They also found 4S RNAs, likely to be tRNAs, and heterogeneously sized polyadenylated RNAs, which were found in mitochondrial polysomes and were thus likely to be mRNAs.

Mapping studies were done in collaboration with Norman Davidson using his latest techniques for visualizing RNA-DNA hybrids by electron microscopy. Norman's laboratory was also just down the hall in the sub-basement of Church building. These studies showed early on that the genes for 12S and 16S rRNA were close to each other in the DNA. Furthermore, these genes were encoded in the Heavy strand of mtDNA, so named because it had a higher ratio of G plus T nucleotides to A plus C nucleotides than the other strand, the Light strand. Later work showed that tRNA genes were dispersed around the genome, on both strands. In addition, tRNA genes lay on both sides of the 12S and 16S rRNA genes, and between them, as well (1972b).

In 1970, Giuseppe and Barbara went on sabbatical leave to the laboratory of Boris Ephrussi in Gif-sur-Yvette. They lived in Paris for a year, commuting to work in the Paris suburb. They were there to learn techniques of somatic cell fusion and to spend time in France, a country of which both of them were very fond. Giuseppe had to travel back to

Pasadena several times, leading to a strain on the marriage, which ended a year after their return to Pasadena. Happily, Giuseppe and Barbara remained friends.

Back in Pasadena, exciting and fruitful projects on mitochondrial biogenesis proceeded on several fronts. Giuseppe and his group demonstrated that mitochondria multiplied by growing larger and dividing (rather than by *de novo* synthesis) and that the mitochondria did this continuously throughout the cell cycle (1977). They demonstrated also that mtDNA in HeLa cells replicates only at a particular time in the cell cycle, starting in S phase and peaking in the G2 phase (1972a). The same was true for mouse L cells; however, this latter work was never published.

In the area of mitochondrial protein synthesis, Giuseppe and postdoctoral fellow Dennis Lynch found that seventeen different mitochondrial tRNAs could be charged with sixteen amino acids (1976). Postdoctoral fellows Muriel Lederman and Paolo Costantino determined that about a dozen proteins were synthesized on mitochondrial ribosomes. By the late 1970s, Giuseppe and his group had shown that the three largest subunits of cytochrome *c* oxidase were synthesized on mitochondrial ribosomes (1980b). Shortly afterward, the translation product corresponding to cytochrome *b* was identified.[vii]

I joined the laboratory as a postdoctoral fellow shortly after the work on cytochrome *c* oxidase was done. I had met Giuseppe in 1972, we became a couple in 1975, and we were married in 1982. My first project was to identify one or more of the mRNAs encoding the subunits of cytochrome *c* oxidase. I had no success with immunoprecipitation of polysomes, but, with the collaboration of Michael Hunkapiller, I was able to obtain the amino-terminal sequences of the two largest subunits of the enzyme (1981a). This sequence information allowed the identification of the initiator codon in the respective mRNAs.

In the mid 1970s, Giuseppe and his group found that electrophoresis through strongly denaturing agarose methyl-mercuric hydroxide gels, developed by Norman Davidson, could separate the poly(A)-containing RNAs into eighteen discrete species (1978). Restriction enzymes that cut DNA precisely at specific tetra- or hexanucleotide sequences were coming into widespread use in the late 1970s. Using these, Giuseppe and long-time collaborator Deanna Ojala constructed a physical map of mitochondrial DNA that consisted of ordered restriction enzyme fragments. The rRNA genes and the individual gel-purified radiolabeled mRNA species were precisely located in this map by hybridization experiments (1980c, d). This work generated a transcription map showing that most of the Heavy strand was occupied by the genes encoding mRNAs and rRNAs.

Three very long and unstable polyadenylated transcripts of the Light strand were also mapped. There was no evidence of intervening sequences, or introns.

Sequencing

Also, the origin of replication, as defined by the 5' end of the major species of 7S DNA, a semi-stable nascent chain (8), was located on the physical map by hybridization experiments. Giuseppe decided to sequence this region of mtDNA using a chemical method recently developed by Maxam and Gilbert (9), as no one else, despite rumors, seemed to be producing any sequences. In a very short time, his collaborators Steve Crews and Ojala sequenced a two hundred-nucleotide fragment that contained the origin. They sequenced also the 7S DNA, so that its 5' end was mapped in the restriction fragment to the precise nucleotide (1979).

Soon after this work was published, Fred Sanger of the Medical Research Council in Cambridge, England, expressed to Giuseppe his interest in sequencing human mtDNA. Giuseppe was quite pleased that someone of Sanger's stature and expertise was ready to do this. Sanger visited Giuseppe at Caltech to discuss the project with him further. He had developed a powerful sequencing technique that utilized DNA polymerase and chain terminating dideoxynucleotides. Sanger asked for some purified human mtDNA to help start the project. Giuseppe agreed, and in fact sent him a large amount, about 200 μg, of cesium chloride gradient-purified HeLa mtDNA.

Meanwhile, Crews sequenced another fragment by the Maxam and Gilbert method, one that was known from hybridization experiments to contain the 5' end of the 12S rRNA gene and a nearby tRNA gene (1980a). In addition, he determined the sequences of the 5' ends of the 12S rRNA and the 16S rRNA. Thus, Giuseppe and Crews were able to find in the mtDNA sequence the exact start of the 12S rRNA gene. Giuseppe also found, by pen and pad, a sequence upstream that could be folded into a cloverleaf structure. This was the predicted upstream tRNA gene. Unexpectedly, the tRNA gene occurred immediately adjacent to the 12S rDNA sequence with no intervening nucleotides. This was the first finding of "butt-joined" genes, a term used by Fred Sanger and his group to describe many such instances in the human mitochondrial genome.

At the same time, Giuseppe, Ojala, and postdoctoral fellow Julio Montoya started to determine ribonucleotide sequences from the 5' and the 3' ends of individual mitochondrial RNA species. For this work, large amounts of HeLa cells were needed and the RNA had to be free of contaminating nuclear-encoded RNAs. Giuseppe had a system set up

for growing cells in suspension to high density in 3-liter and 5-liter balloon flasks, such that as much as 30 grams of cells could be collected from one flask. (Montoya calculated that during his tenure in Giuseppe's laboratory, he harvested more than 2.5 kg of HeLa cells![viii]) Giuseppe had also developed a way to treat and wash isolated mitochondria to make them free of extra-mitochondrial nucleic acids, following a suggestion by post-doctoral fellow Manfred Albring to use micrococcal nuclease. As a result, the individual mRNA species, after resolution through denaturing gel electrophoresis, could be isolated in chemically pure form.

Sanger's group sent partial sequences of bovine and human mtDNA to Giuseppe as the data came in, before publication. The RNA sequences that Montoya and Ojala had obtained could then be aligned with the DNA sequence, and the 5' and 3' termini could be mapped precisely. In turn, the RNA sequence data from Giuseppe's laboratory helped Sanger's team interpret their data. This simultaneous effort from the two laboratories culminated in the publication of three articles in a single issue of Nature, one from Fred Sanger's laboratory, which presented the entire human mtDNA sequence (10); and two from Giuseppe's laboratory (1981b, c), reporting the 5' and 3' terminal RNA sequences of most of the mRNAs, as well as their fine mapping to the genome.

The picture that emerged, based on the DNA and RNA sequences, indicated an astounding economy of coding information and compactness of gene arrangement. There had already been indications of these features in earlier findings, as mentioned above. The mRNAs in several cases started with an initiator codon; there was no 5' leader. In two cases, a single transcript encoded two overlapping reading frames. The majority of reading frames lacked a termination codon; in those cases a UAA termination codon was formed by post-transcriptional poly(A) addition. Most genes were butt-joined to the adjacent genes. This led Giuseppe to propose the punctuation model of mtDNA transcription, a model in which polycistronic transcripts are processed by precise clipping and concomitant CCA addition or poly(A) addition to yield mature tRNAs and mRNAs, respectively (1981c)).

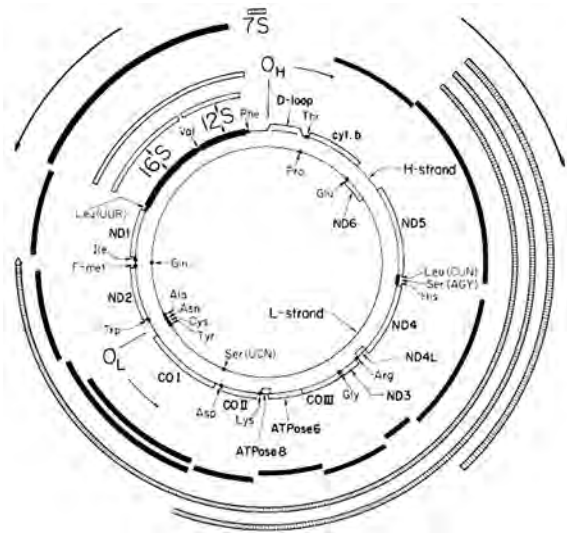
Thirteen reading frames were recognized in the mitochondrial genome. Some of the reading frames were identified by their similarity to genes already known in yeast or by their correspondence to known amino acid sequences from mammals, namely ATP synthase subunit 6 (ATPase6), cytochrome *b*, and cytochrome *c* oxidase subunits I, II, and III. That left eight unidentified reading frames, or URFs.

Unidentified reading frames

Russ Doolittle at the University of California, San Diego, had just developed a new immunochemical reagent, anti-peptide antibodies, that could help Giuseppe match URF to translation product. Doolittle had discovered that antiserum raised against a short peptide could recognize a protein that contained a stretch of amino acids identical or similar to the short peptide (11). Giuseppe telephoned Doolittle and asked if he'd be interested in collaboration. Doolittle himself had been considering approaching Giuseppe. The way Doolittle tells the story, he suggested that Giuseppe come down to La Jolla to meet and talk, to which Giuseppe replied, "We'll be there in two hours."

Thus began a very fruitful collaboration between the two laboratories and the beginning of a lasting friendship between Doolittle and Giuseppe. Over the next few years, the two groups confirmed that all of the URFs were expressed, and they identified the corresponding translation products by peptide-specific antibodies or by protease digestion and peptide analysis.

As to what the function of the URF proteins were, Giuseppe identified the short URF that overlapped the ATPase6 reading frame as being ATP synthase subunit 8, based on its amino acid similarity to the recently identified subunit 8 in yeast (1984). This identification was later confirmed by co-immunoprecipitation experiments (1985b).



Genetic and transcription maps of human mtDNA. From Attardi, 1986b. The circles represent mtDNA. The names of the rRNA and protein-coding genes are indicated. The tRNA genes are indicated as dots on the circles. The arcs outside the circles represent transcripts that were mapped by Giuseppe and his group. The large anticlockwise arrow represents the direction of transcription of the Heavy strand; the Heavy strand transcripts are represented as open or filled-in arcs. The large clockwise arrow represents the direction of Light strand transcription; the hatched arcs represent the mapped light strand transcripts. O_H and O_L indicate the origins of Heavy and Light strand DNA synthesis, respectively.

Giuseppe hypothesized that among the remaining URFs would be subunits of complex I, a very large respiratory chain complex the size of a bacterial ribosome that has rotenone-sensitive NADH dehydrogenase activity. In collaboration with Ian Ragan of the University of Southampton, Joe Hatefi of the Scripps Institute, and with Doolittle, Giuseppe and his group were able to show that all seven remaining URFs are subunits of the respiratory chain complex I (1985a, 1986b). Thus, the informational content of mtDNA in human, and, by extension, animal cells was now completely elucidated. The "1000 Circles in Search of a Function" (an early seminar title of Giuseppe's) were now known to encode 13 protein subunits of the respiratory chain enzymes and the ATP synthase.

After the mtDNA sequence came out, work on its transcription continued with *in vivo*, *in vitro*, and in *organello* approaches. Giuseppe and Yosef Aloni had shown in 1972 that nascent RNA molecules are very long, suggesting that mitochondrial RNA is synthesized in the form of polycistronic precursors. Now Giuseppe and co-workers collected evidence that there were only three transcription units in mtDNA (1983). This low number of transcription units and, correspondingly, of promoters is entirely consistent with the polycistronic nature of the mitochondrial transcripts and with the punctuation model of transcription.

Giuseppe's work on transcription culminated in a paper published in 2005 that tied transcription termination of the rRNA transcription unit to transcription initiation (2005). He and his co-authors proposed that the mtDNA was put into a loop configuration by the transcription termination factor mTERF (1989a). The loop would keep the RNA polymerase, after it had finished synthesizing the rDNA transcript, in the vicinity of the promoter for that transcription unit, thereby facilitating reinitiation of transcription.

Transmitochondrial cell lines and cellular models of disease

An important line of investigation began with the isolation of human cell lines lacking mtDNA by graduate student Michael King (1989b). These cells, named ρ^0 cells, could survive in culture indefinitely if the medium was supplemented correctly. MtDNA could be re-introduced into these cells through mitochondria that contained mtDNA, either by microinjection of purified mitochondria or by fusion with cytoplasts (enucleated cells) or with freshly isolated platelets. The resulting cell is a special kind of "cybrid," having the nucleus of the ρ^0 cell line and the mtDNA of only the donor cell. Human ρ^0 cell lines have become extraordinarily useful genetic tools for the analysis of mitochondrial diseases, especially those caused by mutations in the mtDNA.

The power of the ρ^0 cybrid system was that it allowed one to transfer the mtDNA suspected of harboring a disease-causing mutation and then check the phenotype of the cybrids. A defective respiration rate would indicate that the disease-causing mutation was in mtDNA and not in a nuclear gene for a mitochondrial protein. Giuseppe and I, in collaboration with neurologists in Milan, created just such a cellular model of disease (1991). We made cybrids that carried the mtDNA of a patient with myopathy and other symptoms. Some[ix] of the cybrid clones that we isolated exhibited defective respiration and mitochondrial protein synthesis. These turned out to be carrying a mitochondrial tRNA(Lys) gene mutation that had recently been associated with myoclonic epilepsy and ragged red fibers syndrome (12). Subsequently, cellular models of several other mitochondrial diseases were made in Giuseppe's laboratory and in many other laboratories around the world using these or similar ρ^0 cells.

Aging

Beginning in the 1990s, Giuseppe began work on aging, investigating the role of mtDNA in age-related decline of function. Using fibroblasts derived from people of different ages as mtDNA donors for the construction of cybrids, he and co-workers showed that the age of the mtDNA donor correlated negatively with the respiration rate of the cybrid (1996). This observation was consistent with accumulating evidence in the field of aging research that somatic mutations accumulate in mtDNA with age. Giuseppe and collaborators searched the control region of mtDNA in various tissues for somatic mutations using denaturing gradient gel electrophoresis, a technique that had been developed recently. They found several such mutations (1999). The most intriguing of these, a T-to-G transversion at position 414, occurred in a large proportion, up to fifty percent, of the mtDNA molecules in the fibroblasts of some individuals (1999). Moreover, half the donors in the study over sixty-five years of age had the 414 mutation in their fibroblasts. The significance of the mutation has yet to be elucidated.

Origins of Replication of mtDNA and other topics

In another line of investigation, Giuseppe, together with research assistant Jennifer Fish and graduate student Nicola Raule, made the striking discovery that under certain conditions, a different set of mtDNA replication origins is used (2004). These replication origins are activated when the level of mtDNA is very low and extra rounds of DNA replication per cell cycle are required to restore it to its normal level.

Giuseppe's laboratory made important contributions also in the fields of respiratory control, apoptosis, nuclear chromosome structure, and gene amplification.

Recognition

For his work on mitochondrial DNA transcription, Giuseppe was elected to membership in the National Academy of Sciences in 1984. In 1985 he was named to the Grace C. Steele chair in Molecular Biology at Caltech. Giuseppe was subsequently awarded the Antonio Feltrinelli International Prize for Medicine in 1989, the Gairdner Foundation International Prize in 1998, and the Passano Foundation Award (of Johns Hopkins University) in 2000. The University of Zaragoza conferred the Doctor Honoris Causa to him in 1999. A symposium was organized at Caltech in his honor in 1999, and another, at the University of North Carolina, Chapel Hill, in 2003.

Personality and philosophy

Giuseppe was fundamentally good natured, happy, spirited, and optimistic. He was a man of Old World good manners and an egalitarian sensibility. He greeted everyone he encountered with a smile, from the custodian to administrative assistants to his junior colleagues. He was friendly with his students and post-doctoral fellows, often taking them out to dinner. On holidays he tried to make sure that no one would be alone.

Julio Montoya and Giuseppe Attardi, November 1999, at the ceremony awarding Doctor Honoris Causa to Giuseppe at the University of Zaragoza. (Photo courtesy University of Zaragoza.)

Giuseppe had a classical education, learning Latin and Greek in *liceo*, the equivalent of our high school. For many situations he had an appropriate Latin saying. Two of his favorites were "*In regno caecorum beati monoculi*" (In the land of the blind, the one-eyed man is king) and "*Ex stercore aurum*" (From dung, gold). The latter was often accompanied by a chuckle. He took great pride in his Italian heritage, having attended a university at which the professors and alumni included Copernicus, Fallopius, Galileo Galilei, William Harvey, and Giovanni Battista Morgagni. Giuseppe was fluent in Italian, French, and English, and he knew German well enough to write a paper in that language.

Giuseppe spoke English with a thick Italian accent. However his English grammar and syntax were perfect. He took pride in writing well and put much effort into his manuscripts, editing and re-editing, going over them perhaps a dozen times or more. He usually wrote the introduction and the discussion sections of manuscripts, and he left the writing of materials and methods, as well as the results, to the student or postdoctoral fellow who had carried out the experiments. Giuseppe would write his sections in longhand on a lined yellow pad. He never learned to type or to use a word processor. He was in the habit of always having a yellow pad with him and perhaps also a typed manuscript that he could work on in spare moments. This habit sometimes exasperated family members. The yellow pad appeared on trips to the beach with Barbara, and with Laura and me on camping trips in the Sierra Nevada Mountains, while driving on scenic roads in Kauai, and in the Jardin de Luxembourg in Paris.

Giuseppe's day-to-day participation in laboratory activities was not limited to writing and editing manuscripts, of course. He trained his collaborators, including Barbara, in excellent bench technique. He was technically proficient and had exceptional ability to work precisely with very small volumes and quantities. He stopped doing experiments himself, or working closely with a technician, sometime in the 1970s, except for cell culture experiments; but he was always willing, if not eager, to pitch in at certain stages of an experiment. He particularly liked to break cells, a task that was done with a motor-driven Potter-Elvehjem homogenizer. This took some skill, as the intention was to break the cells by rapid cavitation rather than by shearing (13). We had to hear the indicative "pop."

When Barbara or I needed to make sucrose gradients, he was only too willing to help pour gradients or collect fractions. These were trivial for him, as so much of the early work made use of sucrose gradients. When I first arrived in the laboratory, he showed me a fraction collector that could collect from six sucrose gradients simultaneously. Moreover, there were two or three of such instruments in the laboratory. One could run twelve or more gradients at a time! Such an experiment would not daunt Giuseppe.

Even more than writing and bench work, Giuseppe loved discussing research results and plans with his mentees, the postdoctoral fellows and graduate students. He would invite one into his office, asking him or her to bring the most recent data and pulling out his own light box so that they could look at autoradiographic films, and they would sit and discuss what needed to be done next. He shared his excitement about the work and he provided encouragement and optimism. He tried to inspire his collaborators with one of

his favorite sayings: "*Per aspera, ad astra*." His interpretation of the Latin was, "Through hard work, to the stars." He shared his strong faith that hard work would be rewarded.

Many of Giuseppe's collaborators became lifelong friends. Some, like Julio Montoya and Carlo Morandi, returned to Pasadena on many occasions, including sabbatical visits. Others, like Joel Huberman, Paolo Costantino, and Brian Storrie and his wife Muriel Lederman, stayed in touch by correspondence. It was Storrie who suggested having a symposium in Giuseppe's honor in 1999.

As mentioned earlier, Giuseppe had moved into the space formerly occupied by Dulbecco, in the sub-basement of Church building. The bathrooms were one floor up, in the basement, the coffee vending machine was on the first floor (or later, in the basement), and the secretarial and typing staff was on the first floor. The elevator was excruciatingly slow, encouraging people to use the stairs. Thus, Giuseppe maintained his already strong cardiovascular system by running up the stairs, or at least taking them two at a time, multiple times per day. At the Caltech symposium honoring Giuseppe, Seymour Benzer commented that when Giuseppe telephoned him and asked if he was free for a quick chat, Benzer would look at his watch. Giuseppe would arrive at Benzer's office on the second floor within twenty seconds.

Attardi lab alumni and current members at Gordon Research Conference in New Hampshire, 2004. Left to right: Ian Holt, Jaehyoung Cho, Nicola Raule, Anne Chomyn, Miguel Martin, Giuseppe, Yidong Bai, Min-Xin Guan. (Photo by Eric Schon, courtesy Anne Chomyn.)

Giuseppe returned to Italy at least once a year. The purpose of the trip was usually to give a lecture at a conference or satellite meeting on mitochondria. For a long time, the most important conferences in Europe in the field of mitochondrial research were the Bari conferences, begun in 1965 by Ernesto Quagliariello, a biochemist at the University of Bari, Italy. The Bari meetings brought together the best mitochondrial biologists from Europe and the United States. The meetings were exciting and stimulating for the exchange of the latest, usually unpublished findings in mitochondria

research. I recall one European telling me that these meetings motivated him to do the best research he could so as to be invited back for the next meeting. And, to add to the pleasure of these conferences, the food was always good and the atmosphere congenial. Giuseppe formed several lasting friendships at these meetings.

These visits to Italy were one way that Giuseppe maintained ties with Italy. Over the years, at least eighteen students and postdoctoral fellows from Rome, Padua, Bari, and other cities in Italy came to work in his laboratory. There were also collaborations with clinicians in Milan and Padua, and with geneticists in Bologna. Giuseppe came to be very well known among Italian biologists. As of this writing, he is ranked second, behind Erminio Costa, among deceased Italian scientists by the Hirsch index, a measure based on productivity and continuity of his impact over time.[x]

Giuseppe took the occasion of being in Europe to visit family and friends in Rome and Padua whenever possible. On some occasions, he extended his stay in Europe to go to Vent, in the Austrian Alps, where he enjoyed hiking. Here his stair-climbing conditioning served him well. In his youth he had hiked for days on end in the Alps, stopping at a different *Hütte* every night. He continued to enjoy hiking even to an advanced age.

Giuseppe enjoyed walking, as well. He and I had the habit to take a walk after work and before dinner. This was when we could talk about experiments or more trivial things. We generally walked about two miles. Giuseppe's normal walking speed was fast, but occasionally we race-walked and timed ourselves. Although he was twenty-six years older than I, he was always faster.

Besides hiking and walking, Giuseppe had several other interests outside of science. One of them was classical music, to which he often listened as he worked. He also enjoyed folk and world music. Giuseppe appreciated fine art, as well, and he had a collection of lithographs by Mexican, European, and Japanese artists.

Giuseppe's research program continued to be vigorous until a few years before his death. In the last year of his life, the prostate cancer that he had been living with since 1999, and its treatment, started to take its toll. He was very private about his illness, letting only his family know about it. He exhibited good manners until the end. When a hospice facilitator came to the house for a meeting, a very weak Giuseppe told me to ask her if she would like to have something to drink. He died peacefully at home.

I worked with Giuseppe for more than twenty-eight years and we were married for twenty-five years. I will probably never fully appreciate how working with him and being married to him have influenced my character, my scientific abilities, my appreciation for music and art, my interaction with others, and my view of life. Writing this memoir has given me the opportunity to learn yet more about him, especially his early years, and to experience again the excitement of his research and pride in his accomplishments. I hope that this narrative has done justice to a unique and extraordinary man.

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ENDNOTES

i Arthur Kornberg, MD, Biochemistry at Stanford, Biotechnology at DNAX an oral history conducted in 1997 by Sally Smith Hughes, PhD, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 1998. Available at http://content.cdlib.org/view?docId=kt6q2nb1tg&doc.view=entire_text.

ii Personal communication with M. Cohn by email message, May 11, 2014. The group fully expected to observe that cells produced antibodies of only a single specificity.

iii *Ibid*.

iv Since that time, other immunologists have discovered dual specificity cells—see comments of M. Weigert in M. Cohn, N. A. Mitchison, W. E. Paul, A.M. Silverstein, D. W. Talmage, and M. Weigert (2007). Reflections on the clonal-selection theory. *Nature Reviews Immunology* 7: 823–830—though the clonal selection theory has for the most part turned out to be correct. The dual specificity is referred to as "incomplete or imperfect allelic exclusion."

v Sydney Brenner, François Jacob, and Matt Meselson published their discovery of mRNA in the same issue of *Nature.* See S. Brenner, F. Jacob, and M. Meselson (1961). An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* 190: 576–581.

vi Jerome Vinograd was the originator and developer of density gradient centrifugation. He and Giuseppe benefited mutually from the proximity of their work spaces. Giuseppe's lab provided advice and materials for Vinograd's work on mtDNA, whereas Vinograd's development of a technique to obtain mtDNA in great purity and high yield was very useful to the Attardi lab.

vii C. Doersen and G. Attardi, unpublished results (cited in Chomyn et al. 1985b).

viii Personal communication with Julio Montoya by email message, July 6, 2014.

ix The patient was heteroplasmic for the mutation, both intercellularly and intracellularly. Later work showed that only those cybrid cells in which ≥90% of the mtDNA molecules were mutant exhibited a mutant phenotype.

x http://www.topitalianscientists.org/list_deceased_italian_scientists.aspx.

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