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SEYMOUR BENZER
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A Biographical Memoir by
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Biographical Memoir

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BY RALPH J. GREENSPAN

SEYMOUR BENZER WAS BORN in New York City in 1921. His parents were immigrants who had come to the United States some 10 years earlier from the Jewish *shtetl* of Sochaczew near Warsaw. A true scientific romantic, he was a pioneer in two different fields of biology: the initial studies of the nature of the gene in the early days of molecular biology, and later the launching of a new field that applied mutant induction and other genetic approaches to the study of behavior. In the century that began with the rediscovery of Mendelian units of heredity and ended with the sequencing of the human genome, Benzer's work set two milestones. His early work in bacteriophage on fine structure of the gene defined a pivotal moment in the transition from classical to molecular genetics. His later work in the fruit fly, *Drosophila melanogaster,* launched an entirely new genetic strategy to tackle the complexity of behavior.

Benzer's parents both worked in the Brooklyn garment industry and briefly had their own clothes shop. The only boy of three children, Benzer's earliest scientific ventures included catching frogs and dissecting them with the family clothes-making tools during summers in the Catskills. But it was the gift of a microscope from his uncle on his 13th birthday and the establishment of a "laboratory" in his

basement that really began his scientific explorations, both of which were encouraged by a chemistry teacher and the Chemistry Club at the New Utrecht High School. The first member of his family to go beyond high school, he entered Brooklyn College in 1938 with a Regents Scholarship—all the family's savings for his college tuition having been lost in the 1929 stock market crash. There he majored in physics and chemistry, forgoing biology because the taxonomic approach typical of biology teaching of the day seemed much less challenging. With the encouragement of his physics teachers, he applied and was accepted into the graduate program in physics at Purdue University in Lafayette, Indiana. As the prospect of moving away approached, his father urged him to marry his college sweetheart, Dorothy ("Dotty") Vlosky, who was just completing nursing school, which he did on their day of departure for the Midwest.

As a physics graduate student at Purdue in the period immediately following Pearl Harbor, he came under the influence of the Viennese physicist Karl Lark-Horowitz, who recruited him into a secret wartime project studying the semiconducting properties of germanium, work that foreshadowed the development of the transistor. Because he was doing research related to the war effort, he received a deferment from the draft. When his stipend was raised from $70/month to $120, Dotty could afford to enroll as an undergraduate herself and obtained her bachelor's degree.

During this period, a lab mate lent him a copy of Erwin Schrödinger's *What Is Life?* This book, so effective as a recruiting tool in the early days of molecular biology, framed the challenge of finding the physical basis of the gene. Benzer figured that if one of the giants of quantum mechanics could speculate seriously that the problem of heredity might reveal new laws of nature, it must be challenging enough for physicists to tackle. The romantic notion of exploring

totally uncharted waters appealed to Benzer then and for the rest of his life. He was undaunted by the fact that many traditional geneticists took a dim view of phage, telling Benzer that if he wanted to study genetics, he should work on a "real organism."

Schrödinger's book highlighted the genetic speculations of the young quantum physicist Max Delbrück, who had taken up investigations of bacteriophage as a possible example of an elemental genetic entity. In 1948 while an assistant professor of physics at Purdue, Benzer took Max Delbrück's summer phage course at Cold Spring Harbor and parted ways with physics research for good. He joined the small international community of scientists known as the Phage Group (led by Delbrück and Salvadore Luria and including Alfred Hershey, Leo Szilard, James Watson, and Gunther Stent, among others) and spent as much time away from Purdue in various phage labs as he did being a faculty member. Delbrück served this group as founder, organizer, cheerleader, critic, and even as scoutmaster for its regular camping trips in the deserts east of Caltech. All of Benzer's papers from the phage era end with an acknowledgement to Delbrück "for his invaluable moderating influence."

During his first several years in phage work, Benzer's experiments explored the stages of phage replication. In the back of his mind was a dominant question at the time of whether a phage was itself a gene. The path to the fine structure work began inauspiciously with the testing of some mutants of phage T4 that produced plaques with rough edges (*r* mutants) in preparation for a lab course at Purdue. Finding that some mutants grew on one strain of *E. coli* and not another would eventually be crucial to the experiments (1966). With the dissemination of Watson and Crick's model for DNA structure in 1953, to which Benzer was very receptive, there was a clear need to reconcile the concept of the

gene with the linearity of DNA. In that same year Benzer presented a seminar at Purdue on a review article by the fungal geneticist Guido Pontecorvo entitled "Genetic Formulation of Gene Structure and Gene Action." Pontecorvo framed the problem in the following way:

> [There are] various ways in which a gene can be defined; they are consistent with one another at certain levels of genetic analysis, but not at others…(1) as a part of a chromosome which is the ultimate unit of mutation; (2) as the ultimate factor of inheritable differences, *i.e.,* as unit of physiological action; and (3) as the ultimate unit of hereditary recombination (Pontecorvo, 1952).

The review pointed out that resolution of these issues would require the ability to detect extremely rare recombination events in order to map mutations within the same gene, as well as to construct strains with two closely linked mutations on the same chromosome.

Benzer's work during this period wandered around the questions of phage replication, host range, and phenotypic (plaque morphology) expression. It was decidedly not hypothesis driven. The idea of mapping only began to emerge gradually from these experiments as he noticed and confirmed recombination between mutant loci, confirmed the strain-specific nature of plaque morphology for various *rII* mutants, and also from his correspondence with Alfred Hershey and Gus Doermann, both of whom were assembling linkage maps of T4 phage (Holmes, 2006). In a letter accompanying some T4 stocks that Benzer had requested, Doermann prophetically wrote,

> Sending you my stocks, however, has one condition. This arises from the fact that everyone wants to use genetically known material, but no one is willing to do the more or less thankless and dull job of mapping the markers. There the condition is that you must promise to locate on the T4 map at least two of your independently arising mutants (quoted in Holmes, 2006).

In the spring of 1954 Benzer hatched a plan to use classical genetic mapping to define the functional structure of the gene. There was no way to define the gene biochemically at that time. The discovery of mRNA was still eight years off and gene cloning was not even a glimmer in anyone's eye. So in a return to Pontecorvo's formulation Benzer used traditional genetic recombination to move classical genetics down to the DNA level. His approach was a variation on the *cis-trans* test originally developed by Ed Lewis in *Drosophila* (Lewis, 1951) for assaying whether two mutations produce similar or different phenotypes when they are on the same chromosome (in *cis*) as compared with opposite chromosomes (in *trans*).

Benzer set out to saturate the *rII* region with mutations, a novel concept in its own right, and then to map all of them with respect to one another. He had calculated that he would need to be able to detect recombination events as rare as 5×10^{-6} in order to be able to detect a recombination event between adjacent nucleotides and that this was feasible. Thus, several elements converged to make the experiment work: the ease of selecting the mutant phenotype and the sheer number of progeny that could be generated in phage made the analysis possible down to a level of resolution and degree of saturation unthinkable in *Drosophila*. By taking advantage of the observation that with a high enough titre it was possible to infect a single bacterium with more than one phage, Benzer was able to perform *cis-trans* tests on these otherwise haploid genomes (1955). Practically speaking, the work involved doing the same experiment over and over: isolate mutations, map them with respect to one another, perform *cis-trans* tests. Working mostly by himself, Benzer described it as "Hershey heaven" in reference to Alfred Hershey, who was able to do the same experimental procedure repetitively and continue to obtain useful data from it.

The result was a physical map of the *rII* region of phage T4 almost to the nucleotide level, from which Pontecorvo's three units of genetic function could be discerned. The units of mutation and of recombination were at the limit of resolution, suggesting that they were at the single nucleotide level. The unit of physiological function, on the other hand, was a long stretch of hundreds of nucleotides with distinct boundaries. These units were defined in the *cis-trans* test by the fact that two mutations in the same functional unit would fail to complement in *trans* configuration, whereas two mutations in adjacent functional units would be able to complement in *trans*. In *cis* configuration both types could be complemented by a wild-type chromosome. Thus was coined the term "cistron" for the unit of genetic function, a term that has not stood the test of time as well as the experiments themselves.

Further analysis of chromosomal deletions of various sizes inside, outside, and across the *rII* region, including one that resulted in a fusion of the two adjacent cistrons of *rII* into what he inferred to be a chimeric gene product, allowed Benzer to perform a topological analysis of the arrangement of all these factors (1959). The result supported the conclusion that a functional gene was a linear stretch of DNA with definable boundaries, and that these stretches of DNA were all linked to each other as adjacent pieces of chromosome.

These midcentury findings reverberated back to the time of Alfred Sturtevant's discovery 40 years earlier that the stable Mendelian units of heredity were arranged linearly along the chromosome in *Drosophila,* and also back to Hermann Muller's attempts in the 1930s to grapple with the nature of the gene. Benzer had forged the link between the macro level of Sturtevant's map and Muller's gene concept to the

micro level of the linear structure of DNA, all accomplished by the simple act of performing genetic crosses, beautifully conceived and analyzed. After presenting his results at the 1955 Brookhaven Symposium, Benzer was approached during the break by an elderly man bringing him a piece of cake. Hermann Muller was offering his congratulations.

The work was received as earthshaking from the outset and the awards began to roll in. These would eventually include the Ricketts Award of the University of Chicago, election to the National Academy of Sciences (in 1961), the Canadian Gairdner Award, the Lasker Award, the T. Duckett Jones Award of the Helen Hay Whitney Foundation, the Prix Charles Leopold Mayer of the French Academy of Sciences, the Louisa Gross Horwitz Prize of Columbia University, election to the Royal Society, the National Medal of Science, the Thomas Hunt Morgan Medal of the Genetics Society of America, the Wolf Prize for Medicine, the Crafoord Prize of the Royal Swedish Academy of Sciences, and the Mendel Award of the Genetical Society of Great Britain. By the end of his life when he began receiving prizes for his neurogenetic work, the list encompassed almost every prestigious prize for the life sciences in existence (such as the International Prize in Biology of Japan, the Passano Award, the National Academy of Sciences Award in the Neurosciences, the Bower Award for Brain Research, a second Gairdner Award, the Gruber Award, and the Albany Medical Center Prize.) His impish humor also leaked out occasionally during his many speaking engagements. In one such incident he described the discovery of a new drug, bubbamycin, that reversed the flow of genetic information: from protein to RNA to DNA (a pun on the Yiddish phrase *bubba meises* that literally means "grandmother's stories" and figuratively means "old wives' tales.") The joke preceded by 10 years the discovery of reverse transcriptase.

As the golden decade of early molecular biology unfolded (1953-1963), Benzer's research became more biochemical and resulted in additional seminal contributions. One of these was the demonstration that the aminoacyl tRNA synthetases, the enzymes that attach the correct amino acid to each tRNA molecule, are the actual translators of the genetic code (1963). This was shown by chemically modifying cysteine to alanine after it was already linked to its tRNA, and observing in vitro that alanine was now incorrectly inserted into a hemoglobin polypeptide where cysteine should have been. Another of his studies from this period demonstrated the degeneracy of the genetic code by correlating the different insertion sites of leucine into hemoglobin (again in vitro) with specific leucine condons (1965).

At this point his erstwhile mentor, Max Delbrück, needled Benzer over the number of papers he was now writing; his publication rate had gone from less than one per year to three or four per year. Delbrück wrote, "If I gave them the attention his papers *used* to deserve, they would take all my time" (1966). The comment hit home and encouraged a nascent interest that Benzer had been cultivating on the side in his Purdue lab. For the previous few years he and his technician, Mary Lou Pardue, had been dissecting and sectioning brains from various animals from fruit flies to cows. (As part of Benzer's phylogenetically promiscuous taste for food, some of these were taken home and cooked for dinner afterward.)

Benzer's interest in genetic influences on the brain was prompted by several events. He had been intrigued with the findings of the advertising man turned psychologist James McConnell who claimed in 1962 that RNA isolated from trained *Planaria* could be administered to untrained *Planaria* and the behavior transferred to them. This finding spawned

a bubble of experiments in rats and reports in top journals, all of which confirmed McConnell's basic findings. The bubble finally burst when it was shown that all of the results were artifacts, unduly influenced by wishful thinking. The excitement at the time, however, is readily understandable as a possible molecular mechanism for learning and memory. There was much speculation as to whether there might be a neurogenetic code. Benzer even tried his hand at conditioning *Planaria,* but gave up when he found that electric shock split the worms in two. A second influence was reading *The Machinery of the Brain* (Wooldridge, 1963). Wooldridge had been director of electronics research at Hughes Aircraft and then one of the founders of the aerospace company TRW. In his book Wooldridge laid out a Schrödinger-like challenge to explain the workings of the brain in terms of physics and chemistry. The third influence was Benzer's observation that his second daughter, Martha, totally differed in personality from his first, Barb, despite the apparent lack of change in his and Dotty's behavior as parents.

The catalytic event in Benzer's change of research was a sabbatical year in Roger Sperry's lab at Caltech in 1965. His initial project was to test the effect of phage mutagens on the wiring projection of the frog's retinal ganglion cells onto its optic tectum. The specificity of neuronal wiring was Sperry's signature system at the time, based on the evident fidelity with which a rotated eye would reconnect with the brain. The prospect of using mutagens in this system appeared to Benzer to be an avenue into molecular mechanisms underlying brain function. Unfortunately, the dose required to see any effect was also the dose at which death ensued. But Benzer was undaunted. With encouragement from Caltech's drosophilist Ed Lewis, he began experimenting with fruit flies and their phototactic behavior.

Fruit fly phototaxis had a long research history, going back to the original pre-Morgan fly lab of William Castle at Harvard, but no one had ever tried to induce new mutants to study behavior. This was Benzer's innovation: to take the power of genetic analysis as practiced in phage and bacteria and bring it to bear on the problem of behavior in *Drosophila*. He published his first paper on fly behavior in 1967, the same year he joined the faculty at Caltech, and the field of fly neurogenetics was launched (1967). It had the requisite romantic appeal for Benzer: a problem for which the contours of a solution could not yet be seen. And in a further echo of his earlier romantic quest, traditional neurobiologists told him he was crazy to think that genetics would have anything to contribute to the study of the brain.

For the next 40 years, until his death, Benzer would attract bright scientists both young and old to his lab to explore new areas of fly behavior, neurobiology, and (later on) aging (reviewed in Greenspan, 1990; Weiner, 1999). Among them were most of the founders of what now constitutes the field: Yoshiki Hotta, Obaid Siddiqi, Ron Konopka, Chip Quinn, Jeff Hall, Lily and Yuh-Nung Jan, Yadin Dudai, Don Ready, Tadmiri Venkatesh, Utpal Banerjee, Larry Zipursky, Alberto Ferrus, Mark Tanouye, Barry Ganetzky, Chun-Fang Wu, and Nancy Bonini, among others. No behavior was too far out to be tried, no idea too crazy to entertain. Is there a neurogenetic code? Is there one gene per synapse? Are there such things as behavioral genes? In this precloning, presequencing era, the identities of most genes were still a mystery. If Benzer's phage work was laserlike in its penetrating focus, his fly work had the character of a fountain with streamlets flying off in all directions. In 1973 he wrote an article for *Scientific American* entitled "Genetic Dissection of Behavior," which helped lure many into the nascent field.

Many mutants and genetic approaches that anticipated or started new fields came out of this first decade at Caltech: the circadian rhythm mutant *period* (1971), the neurodegeneration mutant *drop-dead* (1972, 1993), the learning mutant *dunce* (1976,1), the cell fate mutant *sevenless* (Harris et al., 1976), the mapping of behavioral defects to specific sites and cells in the nervous system (1972, 1976,2; Kankel and Hall, 1976; Hall, 1977), and the neurophysiological analysis of mutants (1976,4; 1978; Jan et al., 1977).

As the lab grew bigger there were regular outings to try new restaurants. Where Delbrück had led Phage Group camping trips to the deserts east of Los Angeles, Benzer modified the tradition by leading culinary explorations of greater LA. The more phylogenetically and anatomically diverse, the better, especially if choosing the menu item while it was still alive in its tank was part of the experience. Benzer was also a regular visitor to the area art museums and openings. But behavior remained his prime interest and it extended well outside the lab. He took a keen interest in what we humans do, both normal and aberrant, to the point of attending much of the nine-month murder trial of the infamous Charles Manson at the LA county courthouse.

In the lab's second decade eye development became the principal topic of research, following a seminal study of the dynamics of retinal development in the fly (1976,3). The field was becoming established and Benzer mused that this was the greatest danger to a field, as measured by the founding of a "Journal of…" and an "International Congress of …" The same decade, however, also saw the loss of Benzer's wife, Dotty, to cancer. Some years later he married Carol Miller, a neuropathologist from the University of Southern California, with whom he had a son, Alex.

During his final decade, Benzer turned to the study of neurodegeneration and aging, where he continued to explore new territory. A series of long-lived mutants were isolated, starting with the G protein coupled receptor *methuselah* (1998), as well as a spate of neurodegenerative mutants (1997;1,2, 1999; 2000, 2002). But behavior and fly psychology were never abandoned. Mutants affecting thermo- and hygrosensation were isolated (1996), as was a nociceptive mutant dubbed *painless* (2003), and studies were initiated on feeding behavior (2006).

Benzer was an active and insatiably curious scientist to the end. He pursued science for its own sake starting at a time when it paid so poorly that there was no other reason to go into it. More importantly, he pursued questions whose answers were not at all visible, and for which there was no guarantee of obtaining any results at all. Benzer's accomplishments are emblematic of the half-century during which he worked, an era that saw the problem of the physical basis of the gene solved and the tangled relationship between gene and behavior seriously addressed.

SOURCES FOR THIS article, unless otherwise cited, are conversations with S. Benzer and with former members of his lab.

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