BIOGRAPHICAL MEMOIRS

Charles Yanofsky

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Elected to the NAS, 1966

A Biographical Memoir by Eric U. Selker and Philip C. Hanawalt

Charles Yanofsky was a leading pioneer in the field of molecular genetics, which emerged shortly after World War II. Although trained as a biochemist, Charley was fascinated by genetics and recognized that the approaches of biochemistry would be essential for solving fundamental problems in genetics, such as the structure and function of genes. In the introduction to his Ph.D. dissertation, he stated that his studies represented the first stages in "the elucidation of the nature and mechanism of gene action."[1] Consistent with this long-term goal, decades later his signature advanced course at Stanford was titled "Gene Action" and touched on multiple topics that he personally contributed to during the development of molecular genetics as a discipline. Charley recognized and appreciated that he lived in an exceptional time to decipher gene action. His early work focused on the genetics and biochemistry of tryptophan biosynthesis in Neurospora, leading to discoveries important for the elucidation of the genetic code, and demonstrating the colinear relationship between the structures of genes and proteins. His work on missense mutations and their suppression contributed to our understanding of mRNA function and translation. His later work revealed new features of gene regulation in bacteria, most notably transcriptional attenuation. Some attributes of Charley that we feel were important for his success, in addition to his brilliance, include his extraordinary imagination, leading to great insights; his industrious nature and awesome efficiency, both in the laboratory and at his desk; and his competitive nature coupled seamlessly with his warm and generous personality.

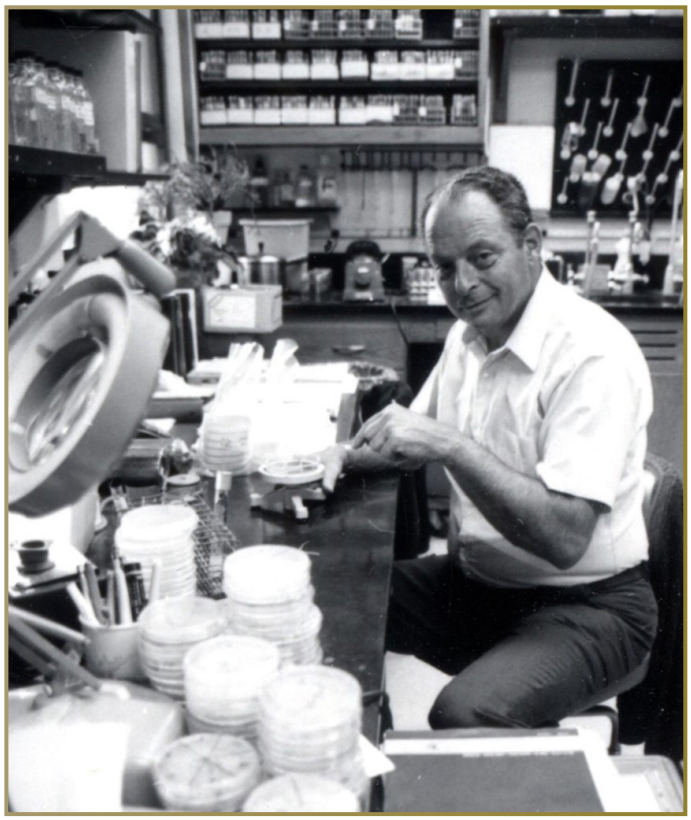

Figure 1 Yanofsky in his lab in July 1985. *Photo coutesty of Chuck Painter; Stanford News Service.*

Personal History

Charles Yanofsky was born April 17, 1925, in New York City to Frank and Jennie Yanofsky, whose families had emigrated to the United States to escape antisemitism in the Russian empire.[i] (Their names in the "old country" were Efraim and Schenike, respectively.) Frank was nine years old when he arrived in 1895, and although his formal education ended with high school, as did that of Jennie, he owned and operated a shirt-manufacturing factory in the 1920s before losing it during the Great Depression. Charley was the youngest of three children, and he credits his sister, Thelma, as a role

model. He later reflected that one of her boyfriends gave him a chemistry set that he enjoyed, for example to make explosives. Charley's son, Marty, remembers that his father was very close to both his brother, Artie, and his sister. Charley and Thelma shared a passion for all things Gilbert and Sullivan. Thelma evidently memorized every line from the shows, and Marty has fond childhood memories of his father at the piano playing familiar tunes from Gilbert and Sullivan. Artie was a decorated military veteran who was taken prisoner by the Nazis at the Battle of the Bulge, in which Charley also served.

Charley was interested in natural history and chemistry from an early age, and he credits an exceptional junior high school biology teacher for opening his eyes to exploratory science.[2] He reflected that he was also fortunate to attend the new, and highly selective, Bronx High School of Science, where he became most excited by biochemistry and genetics, fields that were essentially distinct at the time. Charley carried out his first research at home using Drosophila strains purchased from Cold Spring Harbor Laboratory and as a high school junior applied for, and was awarded, a grant from the American Institute of Science Laboratory, which enabled him to spend the summer generating mutants. He graduated from high school at age seventeen. His parents could not pay tuition at a private college, so he attended the City College of New York (CCNY) concentrating on biochemistry until he was drafted into the military and selected to become a cannoneer. Like his brother, Charley fought in the Battle of the Bulge in December 1944. After a month living in foxholes without adequate clothing, his legs became severely frostbitten, and he spent the last few months of the war in a British hospital. (Of course that may have saved his life.) As a disabled veteran, he was eligible to continue his education under support from the G.I. Bill. He initially applied to Johns Hopkins University and the University of Illinois. But every university was flooded with applications, and he was not admitted to either school, so he went back to CCNY.

Charley noted in his autobiographical review that his departmental chair at CCNY introduced him to the "one gene/one enzyme hypothesis" developed by George Beadle and Edward Tatum, and he became "hooked" and convinced that one needed to use biochemistry to investigate genetics.[3] He applied to Yale University for graduate studies with Tatum and to the California Institute of Technology (Caltech) to work with Beadle. Although he was not accepted at Caltech, he was happy to go to Yale, which was not too far from New York City, where his fiancée, Carol, was finishing her studies. When Charley arrived at Yale, he learned that Tatum had moved back to Stanford, but he was happy to join the exciting group of David Bonner, who stayed at Yale to carry on the legacy of Beadle and Tatum. David initially assigned Charley to work on niacin/tryptophan biosynthesis, but in his third year he changed projects to select an enzyme to address the one gene/one enzyme hypothesis.

Scientific Contributions

Yanofsky's scientific accomplishments are particularly impressive in the context of when they occurred. For example, when he was in graduate school in the late 1940s, it was not yet known that proteins were linear arrangements of amino acids, and it was not even known that DNA was the genetic material. Charley's first task in Bonner's lab was to identify intermediates that accumulated in mutants blocked in the tryptophan-niacin pathway. Coincidentally, one compound that he discovered was a derivative of kynurenine, which Beadle and Tatum apparently worked on fruitlessly in Drosophila, leading them to switch to Neurospora.[ii] Charley's work with mutants defective in steps in the tryptophan pathway, and on unlinked "suppressor" mutations, led to important progress in understanding gene action. He showed that suppressors were frequently allele-specific and restored synthesis of the otherwise defective gene product. In 1955, in an exciting symposium where he met Seymour Benzer (with whom he later shared the Lasker Award and other significant prizes), Charley described his genetic, biochemical, and immunological data that ultimately led him to suggest that suppression may result from misreading the genetic code. Using antibodies that he made to purified tryptophan synthase, Charley was able to show that his suppressible mutants, and suppressed strains, produced immunologically cross-reacting material, leading to the conclusion that they were missense mutations, thus setting the stage to correlate the gene and protein changes. Charley's interest in suppression continued for many years, and importantly, his student, Stuart Brody, became aware of the developing role of tRNA in translation and postulated that mutationally altered tRNAs might be responsible for missense suppressor "mistakes" in protein synthesis. Between games, Charley and tennis partner Paul Berg devised an elegant experiment (ultimately carried out by John Carbon in Berg's lab) to demonstrate that missense suppression was indeed the result of altered tRNAs.[iii]

Colinearity

Even after it was clear that DNA was the genetic material, it was not trivial to show that DNA and proteins are co-linear. After all, sequencing technologies had not been developed. When Yanofsky was convinced to move from his first faculty position at Western Reserve University to Stanford University in 1958, his primary focus became to establish gene-protein colinearity. After Vernon Ingram demonstrated by amino acid "fingerprinting" of proteins that a defect in hemoglobin was caused by a single amino acid change, Charley was quick

to recognize that an analysis of amino acid changes could provide insight into both the genetic code and the question of whether genes and proteins were colinear. He eventually turned to *E. coli* as a model system because of some advantages over Neurospora and developed elegant and efficient methods to generate a fine-structure genetic map of the *trpA* gene. He then related the mutations to amino acid changes detected in tryptophan synthase using fingerprinting methods similar to those employed by Ingram, eventually demonstrating colinearity. Yanofsky's group used missense mutants to demonstrate colinearity, whereas Sydney Brenner's group carried out complementary studies in which nonsense mutants of phage T4 were mapped.[3]

Attenuation

Charley and others realized that gene expression is an "expensive" enterprise for a cell or organism, necessitating gene regulation, but the prevailing view prior to their discovery of attenuation was that regulation of genes such as in the *trp* operon was simply a result of the action of a repressor in the promoter/operator region. Only when they found regulatory mutations between the promoter/operator region and the coding regions, (i.e., in the "leader" region of the mRNA) did Yanofsky and his students realize that the *trp* operon is subject to a mode of regulation independent of the repressor. Careful studies of mRNA levels and on features of the leader region revealed a conditional transcription termination site at the "attenuator." Remarkably, Charley and his associates had discovered that the decision to terminate at the attenuator involves alternative RNA secondary structures that form depending on whether-or-not a fourteen-residue leader peptide, with a series of Trp codons, is translated. When the level of charged $tRNA^{Trp}$ is low, an "anti-terminator" RNA structure forms (first recognized by Charley's student Frank Lee), leading to up to a sixfold increased production of *trp* mRNA. This may have been the first demonstration of involvement of RNA secondary structure in the control of gene expression.

Other Studies with the *E. coli trp* System

Although attenuation "stole the show," Yanofsky's curiosity and his thorough and insightful nature led him and his colleagues to discover and investigate other regulatory mechanisms operating to optimize cellular efficiency, including feedback inhibition of enzyme activity. His group also carried out detailed studies on the structure and function of the Trp repressor and discovered "translational coupling." This discovery resulted from the realization that the coding regions of two pairs of genes in the *trp* operon, *trpE-trpD* and *trpB-trpA*, both of which encode proteins forming complexes, have overlapping stop and start codons (UGAUG). Yanofsky and his colleagues tested the possibility that this resulted in translational coupling between the pairs of proteins, and they found that indeed it does; when translation of the upstream protein was compromised, translation of the downstream open reading frame was also markedly reduced.

Figure 2 David D. Perkins and Charles Yanofsky at 2004 *Neurospora* meeting. *Photo courtesy of M. Sachs, Texas A&M.*

Neurospora Revisited

After Charley consented for one of us (ES) to work in his lab on a eukaryote, as long as it had something to do with tryptophan,[4] we surveyed what was known in model systems (such as yeast, algae, plants, Neurospora) and decided that the fungus *Neurospora crassa* would be most appropriate. Of course, Charley had worked with this organism early on, and I was familiar with it from my undergraduate research. Additionally, David Perkins, the modern "father" of Neurospora genetics, was right next door, and he and his lab were eager to help.[iv] For example, while we were seeking permission to do recombinant DNA research with Neurospora, David constructed strains that might be useful to reduce aerial dispersal of the organism. I was the only one working on Neurospora in Charley's lab in the late 1970s, but before I departed Charley hired a postdoc to continue and expand my studies, and within a few years nearly half of his lab people worked on Neurospora projects, all benefiting from productive interactions with Perkins. Charley finally limited the number of his graduate students and postdocs working on Neurospora, but his cumulative "crop" of fungal workers, which ultimately numbered close to two dozen, significantly stimulated molecular and genetic research with filamentous fungi worldwide. The Yanofsky lab tackled a variety of interesting problems, including gene organization and evolution, gene regulation during sexual and asexual development, mating type gene organization and function, and photobiology. At the same time, members of the group developed technologies to bring Neurospora molecular biology into the modern era, most notably with improved methods for DNA-mediated

transformation, generation of gene libraries, gene cloning, chromosome characterization by pulse-field electrophoresis, and synchronization of development.

Additional Work and Activities

Whereas some members of the Yanofsky group pioneered molecular genetic studies with Neurospora, others continued interesting molecular genetic studies with prokaryotes. For example, investigations with *Bacillus subtilis* revealed striking differences, relative to *E. coli*, in regulatory mechanisms controlling the *trp* pathway. Although the *B. subtilis trp* operon includes a leader sequence expected to form alternative structures, unlike the situation in *E. coli*, the leader does not encode a peptide that can sense $tRNA^{Trp}$. Instead, a novel protein, TRAP, binds to the anti-terminator region of the leader RNA when tryptophan levels are high, allowing transcription termination and also inhibiting translation initiation in several *trp*-related coding regions. In addition, uncharged $tRNA^{Trp}$ plays a role in TRAP action. Yanofsky and colleagues also discovered additional attenuator mechanisms controlling the tryptophan degradation operon of *E. coli*, *tna*, and the *trp*-related *yczA-ycbK* operon of *B. subtilis*. The bottom line is that attenuation mechanisms controlling *trp* gene expression in *E. coli* and *B. subtilis* are remarkably distinct, highlighting the value of their thorough analyses.

Service, Honors, and Awards

Charley earned numerous honors over the course of his career, including election to the American Academy of Arts and Sciences, the National Academy of Sciences, and the American Academy of Microbiology in the United States. He was also elected a Fellow of the European Academy of Sciences and the Deutsche Akademie der Naturforscher Leopoldina, a Foreign Member of the Royal Society, and an Honorary Member of the Japanese Biochemical Society. Stanford University, where he was the Herzstein Professor of Biology from 1967 through 2000, selected him for their H&S Dean's Award for Lifetime Achievements in Teaching in 2003, and he received honorary doctorates from the University of Chicago and Yale. The American Heart Association selected him for its Career Investigator grants annually from 1969 to 1995. His many awards include the GSA Medal and GSA Thomas Hunt Morgan Medal, the Townsend Harris Medal from CCNY, the Wilbur Cross Medal by Yale University and the National Medal of Science by Pres. George W. Bush. Other significant awards include the Lederle Medical Faculty Award, the Eli Lilly Award in Bacteriology, the U.S. Steel Award in Molecular Biology, the Howard Taylor Ricketts Award, the Albert Lasker Award in Basic Medical Research, the Selman A. Waksman Award in Microbiology, the Louisa Gross Horwitz Prize, the Mattia Award of the Roche Institute, the Canada Gairdner International Award, the Passano Award, the William C. Rose Award of the American Society for Biochemistry and Molecular Biology, and the Abbott-ASM Lifetime Achievement Award. Yanofsky also served his academic community as president of the Genetics Society of America in 1969 and the American Society of Biological Chemists in 1984.

Concluding Remarks

Charley loved learning and was not satisfied with less than thorough investigations. In a 1991 editorial discussing the continuing importance of research on prokaryotes, Charley stated "…I believe that the proper practice of science demands intellectual and experimental commitment until the process under scrutiny is thoroughly understood. We should discourage flitting from one project to another."[5] He was critical of the temptation of investigators, granting agencies, and publishers to favor new and glamorous projects, sometimes leading to superficial skimming of exciting new research areas. Indeed, although he was as interested as others in new and exciting findings, a survey of his career accomplishments reveals that he showed the uncommon discipline required to solve problems thoroughly. No one seems to remember Charley himself ever fretting about, or even mentioning, grant renewals, but his editorial revealed that he had an early appreciation of new pressures on investigators that might limit or discourage thorough studies in the future.

Mike Manson, a former student of Yanofsky, collected comments from colleagues for a tribute to Charley a few years after a meeting to celebrate his seventieth birthday, Gene Action '95.[6] The volume includes remembrances and observations on such topics as Charley's scientific thoroughness and tenacity, his phenomenal efficiency, and his exceptional success as a role model. Mike noted that mentoring was most intense at his weekly individual meetings with lab members. I (ES) remember these meetings starting with Charley just saying "So?" or "What's new?" and then quickly moving to a closed-door brainstorming session, only interrupted by the delivery of his iced tea by Virginia "Ginny" Horn, his super technician. Remarkably, because of his phenomenal abilities, on top of running a large successful lab and other responsibilities (teaching, service, etc.), Charley managed to carve out time for himself to work in the lab. He also managed to stay on top of every aspect of his lab, scientific and otherwise. I recall him getting down on his knees to grease shakers before leaving for a few months on sabbatical in the mid-1970s. He also always seemed to find time to give detailed, and nearly immediate, feedback on writing, ideas, and talks.[v] Charley loved science and was competitive, but he it made it clear, as in his talk at Gene Action '95, that people—his colleagues and his family—were of paramount importance to him.[vi]

Charley was also a dear friend and supportive colleague for me (PH) throughout my career at Stanford. I stopped in to meet him upon joining the faculty in 1961 to find him in a crowded basement lab in Jordan Hall, with the names of George Beadle and Ed Tatum still on the lab bench drawers and a tunnel carved into the ground to accommodate his amino acid analyzer. His generosity and excitement in communicating science were immediately apparent. I marveled at his humble manner, as he and his well-mentored students contributed world-class science for well over half a century. He endeared himself to his students and postdocs by his example and the manner in which he challenged them to do their best work. He was also an incisive but gentle critic of student presentations in our weekly molecular biology journal club and research seminar series. His lectures in the core molecular biology course were delivered with precision and clarity; he spoke slowly but did not repeat himself. Students needed full attention to every sentence or they would miss important information. Charley never diluted his dedication to research and teaching with a department chairmanship or other administrative positions, but he was one of the most supportive colleagues for those of us who did. Charley was the primary role model for both of us (ES and PH) on how to be an effective professor. He is remembered with warmth and immense respect by all of us whose lives he touched.

Notes

i Yanofsky provided us with a rich source of autobiographical material, most notably in the form of an interview as part of the Genetics Society of America's "Conversations in Genetics" project, headed by Shelley Esposito (see https://youtu.be/1s3ZyUDlid4) and in his 2001 article in the *Annual Review of Biochemistry*. Readers are also referred to an obituary published in the *Stanford Report*: Stanford geneticist Charles Yanofsky dies at 92, March 16, 2018; https://news.stanford.edu/stories/2018/03/geneticist-charles-yanofsky-dies-92.

ii While in the Yanofsky lab, I (ES) noticed that Charley's graduate school colleague and friend, Gabriel Lester, had demonstrated that kynureninase is highly regulated in Neurospora, leading me to try to isolate the gene. I outlined my plan to Charley, which was to select for its expression in *E. coli*, and when I asked him how I might obtain some kynurenine, he pulled a vial of the compound from his desk drawer, which he had apparently synthesized as a graduate student! Yanofsky gave the first Gabe Lester Memorial Seminar at Reed College soon after I became a biology major there. Ironically, although Lester continued to work with Neurospora until his untimely death, he apparently encouraged Charley to switch from Neurospora to *E. coli*, which Lester had used in Bonner's lab.[7] After Charley abandoned Neurospora because of limitations studying the biochemistry of tryptophan biosynthesis (e.g., because Neurospora is loaded with proteases), he worked almost exclusively with prokaryotes until I brought Neurospora back to his lab in 1975.

iii After first hearing about molecular cloning in the Lester Lecture by Charley's friend and tennis partner, Paul Berg (who later shared a Nobel prize for his role developing molecular cloning), I (ES) was excited when Charley offered me an opportunity to clone and characterize the Salmonella *trp* operon during my first year of graduate school in 1975. It was fun doing some of the first recombinant DNA work with our homemade restriction enzymes and DNA ligase, but I couldn't resist asking Charley if he would mind if worked in his lab on gene regulation in a eukaryote. Surprisingly, he was agreeable as long as my project had something to do with tryptophan.[8] I tried to comply but had more failures than successes, leading me to work on some projects unrelated to *trp*. Frankly I had hoped that Charley forgot about this condition, but he brought it up when he introduced me for my thesis defense.

iv See photo below of David and Charley at the 2004 Neurospora meeting at Asilomar (courtesy of Matthew Sachs, Texas A&M).

v For example, in response to a draft of my first NIH grant proposal in 1985, Charley wrote:

> Dear Eric,
>
> I read your proposal. Overall it is excellent, except for the Specific Aims. I made a few penciled comments here & there in the body of the proposal but the enclosed yellow sheet has my specific comments on Aims. The aims do not do justice to the proposal. Since this is the first summary of the objectives it is the most important. It is the first section the reviewers read after the summary & it is always used as the basis for writing a summary of a proposal. Yours should be totally rewritten. It should give essential background information concisely & understandably—it should present the novel features of your approach, it should state what you hope to accomplish. The aims should be realistic e.g. in your item 7 there is no reason stated why you think either a) or b) will give you methylation deficient mutants.
>
> Phone if you have any questions.
>
> Best regards,
>
> CY
>
> PS: Everyone here has the flu—I hope you can read my writing!

vi The fact that people, especially family and colleagues, were extremely important to Charley was obvious at the Trp conference, held every two years at Asilomar Conference Grounds. Scores of current and former members of his lab met at these Trp meetings to describe their current studies (many of which had their origin in Charley's lab) and to enjoy the company of Charley and his followers, including a small contingent from Charley's graduate student days. After the death of his Ph.D. advisor, David Bonner, to Hodgkin's lymphoma in 1964, at age 48, Charley created a position in his lab for David's wife Miriam, who had been a "no-nonsense" research assistant at Yale in charge of other technicians, including Charley's wife, Carol.[9] Of course, Charley tremendously appreciated that his dedicated wife of forty years provided him with incredible support, helping him in the lab early on and most importantly taking primary responsibility for raising their three sons, Steve, Bob and Marty (all of whom got the science "bug"). After the loss of Carol to cancer and death of his former postdoc and close friend Irving Crawford, Charley married Edna Crawford who also understood and supported his love of science.

References

1 Yanofsky, C. 1951. A study of tryptophan-niacin metabolism in Neurospora. Ph.D. thesis, Yale University.

2 Yanofsky, C. 2001. Advancing our knowledge in biochemistry, genetics, and microbiology through studies on tryptophan metabolism. *Ann. Rev. Biochem.* 70:1–37.

3 Sarabhai A., A. D. W. Stretton, S. Brenner, and A. Bolle. 1964. Colinearity of the gene with polypeptide chain. *Nature*. 201:13-16.

4 Yanofsky, C. 2001.

5 Yanofsky, C. 1991. What will be the fate of research on prokaryotes? *Cell* 65: 199-200.

6 Manson, M. D. 2002. Thanks, Charley. *J. Bacteriol.* 184:2065–2071.

7 Yanofsky, C. 1976. The search for the structural relationship between gene and enzyme. In: *Reflections on Biochemistry: In Honour of Severo Ochoa,* eds. A. Kornberg, B. L. Horecker, L. Cornudella, and J. Oró, pp. 263–271. Oxford, U.K.: Pergamon Press.

8 Yanofsky, C. 2005. Using studies on tryptophan metabolism to answer basic biological questions. *J. Biol. Chem.* 278:10859–10878.

9 Chrispeels, M. J. 2006. David Mahlon Bonner. National Academy of Sciences Biographical Memoirs 88; https://nap.nationalacademies.org/read/11807/chapter/4.

Selected Bibliography

1952 The effects of gene change on tryptophan desmolase formation. *Proc. Natl. Acad. Sci. U.S.A.* 38:215–226.

1963 With S. Brody. Suppressor gene alteration of protein primary structure. *Proc. Natl. Acad. Sci. U.S.A.* 50:9–16.

1964 With B. C. Carlton et al. On the colinearity of gene structure and protein structure. *Proc. Natl. Acad. Sci. U.S.A.* 51:266–272.

1966 With J. Carbon and P. Berg. Studies of missense suppression of the tryptophan synthetase A-protein mutant A36. *Proc. Natl. Acad. Sci. U.S.A.* 56:764–771.

With J. Ito and V. Horn. Amino acid replacements and the genetic code. *Cold Spring Harb. Symp. Quant. Biol.* 31:151–162.

1967 With G. R. Drapeau, J. R. Guest, and B. C. Carlton. The complete amino acid sequence of the tryptophan synthetase A protein (alpha subunit) and its colinear relationship with the genetic map of the A gene. *Proc. Natl. Acad. Sci. U.S.A.* 57:296–298.

Gene structure and protein structure. *Sci. Am.* 216:80-94.

1969 With D. E. Morse. Polarity and the degradation of mRNA. *Nature* 224:329–333.

1975 With K. Bertrand et al. New features of the regulation of the tryptophan operon. *Science* 189:22–26.

1981 Attenuation in the control of expression of bacterial operons. *Nature* 289: 751–758.

1989 With M. L. Springer. A morphological and genetic analysis of conidiophore development in *Neurospora crassa*. *Genes Dev.* 3:559–571.

1993 With P. Babitzke. Reconstitution of *Bacillus subtilis trp* attenuation in vitro with TRAP, the trp RNA-binding attenuation protein. *Proc. Natl. Acad. Sci. U.S.A.* 90:133–137.

2002 With F. Gong. Instruction of translating ribosome by nascent peptide. *Science*. 297:1864–1867.