Charles Yanofsky in his lab in the basement of Jordan Hall at Stanford University in the early 1960s, as his group’s colinearity studies were progressing.

Annu. Rev. Biochem. 2001. 70:1–37

ADVANCING OUR KNOWLEDGE IN BIOCHEMISTRY, GENETICS, AND MICROBIOLOGY THROUGH STUDIES ON TRYPTOPHAN METABOLISM

Charles Yanofsky
Department of Biological Sciences, Stanford University, Stanford, California 94305; e-mail: yanofsky@cmgm.stanford.edu

Key Words suppression, colinearity, attenuation, repression, conidiation

■ **Abstract** I was fortunate to practice science during the last half of the previous century, when many basic biological and biochemical concepts could be experimentally addressed for the first time. My introduction to research involved isolating and identifying intermediates in the niacin biosynthetic pathway. These studies were followed by investigations focused on determining the properties of genes and enzymes essential to metabolism and examining how they were alterable by mutation. The most challenging problem I initially attacked was establishing the colinear relationship between gene and protein. Subsequent research emphasized identification and characterization of regulatory mechanisms that microorganisms use to control gene expression. An elaborate regulatory strategy, transcription attenuation, was discovered that is often based on selection between alternative RNA structures. Throughout my career I enjoyed the excitement of solving basic scientific problems. Most rewarding, however, was the feeling that I was helping young scientists experience the pleasure of performing creative research.

CONTENTS

0066-4154/01/0701-0001$14.00

When I considered the topics I might address in this review, several weighty questions immediately came to mind, such as what attracted me to science, which career goals influenced my decisions, when did I first feel a burning desire to solve a major biological problem, and what pleases me most about my scientific accomplishments? Reflecting on the totality of my contributions to science, what I find most impressive aside from our discoveries are the number and variety of problems we have attacked. I must have recognized early on that progress on any major project was likely to be slow; therefore it would be wise to delve into several simultaneously. To me, it never seemed difficult to identify unsolved basic problems; in the areas of science I was familiar with I felt that our knowledge was grossly incomplete. In the following pages I describe many of the problems we have addressed. It is not possible to cite everyone's contributions, so I trust that my numerous collaborators—students, postdoctoral fellows, visiting investigators, and technicians—will be forbearing in my occasional use of "we" in citing our accomplishments.

My journey as a scientist has been thoroughly enjoyable. I feel fortunate to have had the opportunity to participate in several scientific discoveries. I hope I can convey my enthusiasm to you.

MY EARLY YEARS

What first aroused my interest in science, and why I did choose to become a scientist? Neither of my parents was highly educated. Each completed only high school, and neither had any exposure to the sciences. Both emigrated to the United States in their early teens, escaping with their parents from an anti-Semitic Russia. Like many Jewish immigrants from Europe, they and their families settled in New York City. I was the youngest of three children. The eldest, my sister, served as a role model; in my opinion she was brilliant. Despite growing up in a heavily populated

city, I had a fascination for the organisms around us that I believe initially attracted me to science. In my early teens I developed a passion for collecting butterflies. As a junior high school student in the Bronx I spent many summer afternoons, net in hand, chasing butterflies on the uptown New York University campus, just a few blocks from our home. One summer at camp in Connecticut, I became fascinated by the garter snakes that seemed to be everywhere. When not playing baseball or swimming, I would wander off to observe their strange behavior. I could not understand how they could move, not having legs. I caught a few, and brought one home, much to the astonishment of my normally understanding parents. This snake soon disappeared; family deliberations led us to conclude that our snake had decided to move in with our quieter downstairs neighbors. During this period I spent many hours with a chemistry set that one of my sister's boyfriends had given to me. I built several balsa wood rocket ships, powering them with explosive mixtures I prepared from my chemicals. I also recall visiting a downtown science shop and purchasing all the ingredients needed to culture plants using hydroponics. I grew zinnias to the flowering stage, in old milk cartons, on the roof of our house. An outstanding junior high school biology teacher was the first person to open my eyes to the marvels of exploratory science. She stressed the importance of the hard facts of science, and continually pointed out how little was actually known.

In my last year in junior high school, 1939, I had to select a high school to attend. It was my good fortune that the Bronx High School of Science had just opened its doors; it was about a half hour's walk from my home. I passed the competitive entrance exam and entered as a sophomore in the second year of the school's existence. As I recall, the teachers at the school were outstanding. They were enthusiastic about teaching, knowledgeable about their subjects, and most importantly, they made an effort to convince each of us that nothing could be more challenging or rewarding in life than dedicating oneself to answering the questions of science. Personally, I was "turned on" by both chemistry and biology. I joined what was called the Drosophila Squad, learned microscopy, and taught myself to fix and stain giant salivary chromosomes. I still have one of my photos—it's not bad.

I remember one particularly exciting high school experience. My biology class was taken on a one-day trip to the Cold Spring Harbor Laboratory, where several of the laboratory's scientists described their ongoing genetic research. Upon hearing their presentations I became more fascinated than ever with the questions of genetics. However, I concluded or was persuaded, I do not remember which, that the path to answering the questions of genetics would require application of the approaches of biochemistry. To get some hands-on experience in genetics I purchased a mail-order "genetics kit" from the Cold Spring Harbor Laboratory. It contained genetically marked *Drosophila* stocks, most likely eye-color mutants. The kit came with instructions for performing genetic crosses that would allow the purchaser to verify some of the basic principles of genetics. I performed these experiments with live flies kept in milk bottles, in a closet in the kitchen in our home. How supportive can a mother be! As a high school junior I submitted a research proposal to the American Institute Science Laboratory. The project was

based on the use of ultraviolet light to produce *Drosophila* mutants. The proposal was approved and I spent an entire summer working at the foundation's downtown labs. It was great fun, finally working on something scientific of my own choosing! While in high school I of course read Paul DeKruif's *Microbe Hunters*. It made a deep, lasting impression on me, as it did on so many other youngsters.

Upon graduating from high school in 1942, at age 17, I enrolled as a biochemistry major at the City College of New York. At the time, my parents could not afford to send me to a college that charged tuition. I completed a year and a half of study before I was drafted into the army and sent to Camp Blanding in Florida for basic training as an infantry replacement. Because I had some college experience, I was assigned to become an infantry cannoneer rather than a foot soldier. Upon completion of basic training, in July of 1944, I was sent to a redeployment camp, Fort Meade, which was then supplying replacements for soldiers who were killed or seriously wounded in Europe. I was fortunate in not being sent to Europe immediately; I was assigned to an infantry cannon company of the 75th Infantry Division (then in Kentucky) as it was preparing to leave for Europe. We arrived in early October of 1944. I fought with this division in December of that year, in the Battle of the Bulge. We had been equipped for combat in southern France and were poorly prepared for a winter in Belgium. After a month in combat, living in a foxhole, my legs became frostbitten, and I spent the last months of the war in a hospital in Great Britain. As the war was ending I rejoined my outfit and was stationed first in Germany, and then in France. Given the opportunity to take a one-month leave, I chose to go to Paris to take a course in French language and civilization at the Cité Université. During this leave I visited the Pasteur Institute and viewed some of Pasteur's memorabilia. This visit renewed my yearning to pursue a career in science.

MY DECISION TO GO TO GRADUATE SCHOOL

Upon returning to the United States, I thought it would be wonderful to complete my undergraduate education at an institution outside the city. With the promised security of a monthly check for $105 and other GI benefits, I felt that I could afford to go almost anywhere. I applied to the University of Illinois, where my brother obtained his bachelor's degree, and to Johns Hopkins University, where my sister earned her PhD as an archaeologist. It was my intention to major in biochemistry. I was turned down by both institutions. During this period every university was flooded with applications from former GIs. A prominent biochemist at the University of Illinois did take the time to send me a personal letter explaining their decision. He wrote that on the basis of his evaluation of my undergraduate record at CCNY, I would be well advised to seek a career in some area other than science. His response was correct in one sense: My college record prior to entering the army was not that impressive because my thoughts were elsewhere—on the war—and I found it difficult to concentrate on schoolwork. In the fall of

1946 I returned to CCNY to complete my undergraduate studies as a biochemistry major.

During my senior year I enrolled in an advanced biochemistry seminar course. This was my first opportunity to read original scientific papers. Just prior to the start of this course I met with the chairman of the Chemistry Department, Benjamin Harrow, to inform him that I had decided to attend graduate school, and that I was seeking his advice on where to apply. I told him I was having difficulty making this decision because I was interested in two subjects, biochemistry and genetics. He listened to me intently, thought for a moment, and then pulled down from a bookshelf behind his desk a copy of the biochemistry text he authored. He opened it to a single paragraph, which he asked me to read. This paragraph described the early studies of George Beadle and Edward Tatum on the one gene–one enzyme hypothesis. Harrow recommended that I read some of their papers and present one of them in the biochemistry seminar course I was taking. He also commented that if I found their papers exciting, as he thought I would, I should apply to do my graduate studies with either of these scientists.

I was of course captivated by the Beadle and Tatum papers; they applied biochemical approaches to genetic problems, which is just what I thought I wanted to do. I did apply to the graduate schools at California Institute of Technology and at Yale; I was rejected by Caltech and accepted by Yale. This was very convenient for me because that spring I had become engaged to the woman who was to become my wife, Carol, who had one year of study to complete at Brooklyn College. New Haven was only an hour and a half train ride from New York, so it would be possible to make occasional trips to the city to be with her. As the end of the school year was approaching, I became anxious to begin graduate work and skipped graduation exercises to move to New Haven promptly. When I arrived at Yale I was surprised to learn that Tatum had left, to return to Stanford. I was assigned to his associate, David Bonner. This seemed fine to me, since Bonner shared Beadle's and Tatum's primary interest in using biochemical approaches with *Neurospora* strains to examine the gene-enzyme relationship.

GRADUATE AND POSTGRADUATE STUDIES WITH DAVID BONNER AT YALE

On my first day in the lab in 1948, Bonner handed me agar slants with the fuzzy growth of two Neurospora mutants. He said something like the following: "Each of these slants contains a mutant that is blocked in the conversion of tryptophan to niacin. Each accumulates a compound that supports the growth of a mutant blocked earlier in the niacin pathway. Your assignment is to identify the pathway intermediate that each mutant accumulates." This information, he argued, would help us complete our knowledge of the sequential biochemical reactions involved in niacin synthesis. I immediately became fully engaged in this project, and identified one of the accumulated compounds as a derivative of kynurenine,

N-acetylkynurenine. The mutant appeared to be blocked in the conversion of kynurenine to 3-hydroxykynurenine. It is coincidental that kynurenine introduced me to experimentation with Neurospora. You will recall that it was the difficulty Beadle and Tatum experienced in attempting to identify kynurenine as an eye pigment precursor in Drosophila that is believed to be responsible for Beadle's decision to abandon this organism in favor of Neurospora. The second niacin-requiring mutant, blocked later in the pathway, was shown by Bonner and me to accumulate the intermediate quinolinic acid.

Most members of the Bonner group were studying individual genes and enzymes of Neurospora, or of *Escherichia coli*, hoping to stumble on some combination that would allow them to establish the basic relationship between gene and enzyme. Graduate students Naomi Franklin and Otto Landman were analyzing β-galactosidase mutants of Neurospora, while Gabriel Lester and Howard Rickenberg were studying β-galactosidase mutants of *E. coli*. At the time, 1949–1950, the chemical nature of the gene was not known, nor had the structure of a single protein been established. The most thorough study of the one gene–one enzyme relationship performed to that date, by Clement Markert, dealt with mutations affecting the enzyme tyrosinase in strains of the fungus *Glomerella*. Markert observed that mutations in any one of several genes could alter tyrosinase activity (1). His observations raised serious doubts about the validity of the one gene–one enzyme concept.

While I was a graduate student at Yale, we were often reminded that Joshua Lederberg had completed his graduate studies in our department just a few years earlier. His brilliance, and his extraordinary accomplishment—demonstrating genetic recombination in the K12 strain of *E. coli*—were often brought to our attention. I recall reading Josh's very short thesis, and being overwhelmed by the logic and execution of his amazing studies.

In my second year in graduate school, I too was hoping to identify an enzyme of Neurospora that would be an ideal subject for gene-protein analyses. I set two requirements: First, the enzyme should be assayable in crude extracts, and second, mutants lacking that enzyme activity must have a discernible phenotype. Only a few enzymes satisfied these two criteria. One was an enzyme present in extracts of Neurospora that catalyzed the last reaction in tryptophan formation, the condensation of indole and serine to form tryptophan. This enzyme, tryptophan desmolase, was described by Umbreit, Wood & Gunsalus in 1946 (2). Since two Neurospora mutants required tryptophan for growth but did not respond to indole, mutants C-83 and S-1952, their altered gene and the corresponding enzyme appeared to satisfy my criteria. I set about purifying the wild-type enzyme and assaying extracts of the two mutants for this enzyme activity.

Although deeply involved in my graduate research, I was also taking graduate courses. Most of the biochemistry I learned at Yale was taught to me by Joseph Fruton, chairman of the Biochemistry Department. When I arrived at Yale I met with Fruton to tell him that there was no point in my taking his required biochemistry course because I had been a biochemistry major at CCNY, and I already knew it all! Fruton did not laugh, or seem upset, at least not in my presence. Rather,

he invited me to enroll in his advanced biochemistry lab course, and to take his biochemistry tutorial course, which met for a few hours once a week. I eagerly took both courses. I never experienced anything before, or since, that was as grueling or demanding as Fruton's tutorial. The three of us taking the course spent most of our waking hours preparing for each weekly presentation before Fruton. The effort demanded was extraordinary, but each of us learned so much it was truly a once-in-a-lifetime experience.

The graduate students at Yale at that time were exposed to many great scientists. Dave Bonner made a point of inviting the leading figures in modern genetic research to visit Yale and present lectures on their work. I recall fabulous presentations by some of the field's heroes, Tracy Sonneborn, Salvador Luria, Max Delbrück, and Al Hershey, among others. Jacques Monod, Andre Lwoff, Boris Ephrussi, and Fred Sanger also lectured at Yale while I was there. We all benefited from this exposure to some of the best minds in science. Bonner also invited geneticist Louis Stadler to spend a semester at Yale to present a course devoted entirely to research on the R locus of maize. Stadler was perhaps the leading maize geneticist at the time, and his contributions were comparable to those of Muller with Drosophila. Stadler was convinced that the beads-on-a-string concept of genes and chromosomes was wrong, and that the R locus of maize was complex, genetically. He believed that most independent mutational changes at this locus were distinct, and should be separable by genetic recombination. I took his course, which was also attended by my wife Carol, and by Dave Bonner's wife, Miriam, both of whom were working as technicians for Dave. I was enormously impressed upon hearing the scientific logic that was Stadler's hallmark. Yes, the entire course was devoted to studies on this single locus. As is often the case in such courses, Stadler required each of us to select an unsolved problem in genetics and write a term paper describing how we would go about providing a solution. After some thought, and considerable reading, I selected genetic suppression as my topic, and described what I would do if I were studying its molecular basis. Suppression (restoration of function to a mutant by a mutation in an unlinked gene) had already been observed in genetic studies with mutants of maize, Drosophila, and Neurospora.

While I was taking Stadler's course I was also performing tryptophan desmolase assays with the two mutants of Neurospora, td1(C-83) and td2(S-1952), that were thought to be blocked in the conversion of indole to tryptophan. I showed that neither mutant had demonstrable tryptophan desmolase activity. By coincidence, in one experiment one of my td2 control strains acquired the ability to grow without added tryptophan. Genetic analysis revealed that this growth was due to an unlinked suppressor mutation. This finding, and the paper I had written on the mechanism of suppression for Stadler's course, intensified my interest in this phenomenon. I performed enzyme assays on the suppressed strain and showed that it did have tryptophan desmolase activity (3). In view of this interesting result, other members of the Bonner group isolated additional tryptophan desmolase mutants, which were all subjected to reversion/suppression analyses. Several of these mutants also yielded suppressor mutations, most of which were allele-specific. An

additional finding, which we could not explain at the time, was that some of our mutants appeared to be nonsuppressible. These studies were summarized in what I consider one of my most provocative early papers, entitled "Gene Interactions in Enzyme Synthesis" (4). I presented our findings at an exciting symposium entitled "Enzymes: Units of Biological Structure and Function," at the Henry Ford Hospital in Detroit in 1955. The meeting was attended by most of the world's leading biochemists and geneticists, as well as newcomers to these fields. I met Seymour Benzer for the first time at this meeting, and he and I had several long discussions on issues of common interest. Soon after this symposium ended, I learned of Benzer's outstanding analyses equating genetic map with gene. In my symposium article I describe the genetic, biochemical, and immunological data that led me to conclude that suppression may involve misreading of the genetic template that encodes the enzyme tryptophan desmolase. Messenger RNA, of course, had not yet been discovered. I found that suppressed mutants produce two proteins, one inactive, presumably the encoded mutant protein, and a second one active, believed to be produced as a consequence of mistranslation via suppression.

Just prior to these studies, I performed some exciting immunological experiments with a former graduate student of Dave Bonner, Sigmund Suskind. Sig had left Bonner's lab to do his postdoctoral studies in immunology, with AM Pappenheimer. In his spare time, Sig prepared an antiserum to my partially purified preparations of wild-type Neurospora tryptophan desmolase and showed that the serum inhibited the enzyme activity. We planned to use this antiserum to determine if extracts of mutants and suppressed mutants contain an inactive protein immunologically related to tryptophan desmolase that would titrate out the antibodies that could inhibit tryptophan desmolase. To perform these experiments, Sig came to Yale for a long weekend. To make the tests more exciting I coded the extracts from our strains. Using his antiserum, Sig demonstrated that some of the mutants produced a tryptophan desmolase cross-reacting material, which we named CRM, while others did not (5). All of the suppressible mutants, as well as the suppressed mutants, appeared to produce CRM. This was one of the earliest demonstrations that mutants lacking an enzyme activity could produce an inactive protein immunologically related to the wild-type enzyme. Needless to say, we were quite excited by these findings.

But these studies were not taking me any closer to examining the one gene–one enzyme hypothesis. In fact, I was having great difficulty purifying Neurospora tryptophan desmolase, the enzyme I had selected for my gene-enzyme project. If I couldn't prepare the protein in pure form, how could I determine the amino acid change in each mutant protein? I made a serious effort to identify a more suitable Neurospora enzyme, examining both D-serine and L-serine dehydrases, but was unable to isolate mutants lacking either of these enzymes. Frustration led me to consider switching to a simpler organism, and I began experimenting with *E. coli* and *Bacillus subtilis*. The initial project I selected was to determine if these bacteria synthesize niacin from tryptophan by the same pathway used by Neurospora and most mammals. My isotopic analyses revealed that neither bacterium synthesizes

niacin from tryptophan (6). Subsequent investigations by others confirmed this conclusion; the significance of this early finding demonstrating alternative pathways in nature is described in an article by Penfound & Foster (7).

AS ASSISTANT PROFESSOR OF MICROBIOLOGY AT WESTERN RESERVE UNIVERSITY MEDICAL SCHOOL (1954–1958)

While I was completing my graduate studies at Yale, in 1951, the Oak Ridge National Laboratory was negotiating with David Bonner, hoping to entice him to accept a position in its Biology Department. This invitation was attractive to Bonner, mainly because he felt that Yale did not appreciate him. After some thought Bonner decided to move to Oak Ridge, and he invited Gabe Lester and me to join him. We both thought the world of Dave and were eager to go with him. But negotiations faltered when Bonner initially experienced difficulty obtaining the security clearance that was required of workers at the Oak Ridge National Laboratory. Lester and I then started looking for positions elsewhere, and during a Johns Hopkins' biochemistry symposium, I was approached by members of the Department of Microbiology at Western Reserve University Medical School. I visited the school, both parties were pleased, and I accepted their offer. I moved to Cleveland in 1954. I found the faculty at Reserve to be truly outstanding and strongly oriented toward basic research. When I arrived I learned that I was to be their token geneticist!

As a green recruit, it seemed to me that it would be wise initially to focus on a relatively straightforward project. I temporarily put aside my interest in the gene-enzyme relationship and set out to determine the unidentified intermediates in the tryptophan biosynthetic pathway. I chose an enzymological approach, and planned to examine cell extracts of *E. coli* and its mutants. The intermediates I soon detected were all phosphorylated. This is undoubtedly why they had not been identified previously; phosphorylated compounds do not support the growth of mutants. I selected *E. coli* as my experimental organism for three reasons. First, I could readily isolate mutants that appeared to be blocked in different reactions in the tryptophan pathway. Second, most of my mutants overproduced tryptophan desmolase and presumably the other tryptophan biosynthetic enzymes, when cultured on growth-limiting concentrations of tryptophan. Third, genetic analyses could be performed with this organism. I used a combination of isotopic analyses, enzymology, and intermediate isolation and identification to establish that the previously known early intermediate, anthranilate, initially reacts with phosphoribosylpyrophosphate to form phosphoribosyl anthranilate (8) (Figure 1). This compound is then converted to the intermediate carboxyphenylamino-deoxyribulose-5-phosphate (CdRP), which is then converted to indoleglycerol phosphate (InGP) (Figure 1). InGP serves as the precursor of indole, which we knew reacted with serine to form tryptophan (9, 10) (Figure 1). When I performed these experiments I was in the right place at the right time. Robert Greenberg and David Goldthwait

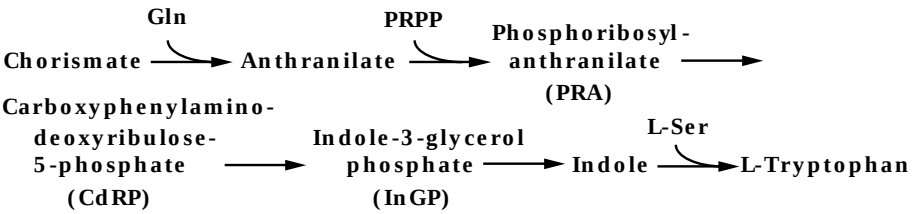

Figure 1 The biosynthetic pathway of tryptophan formation.

of our Biochemistry Department were just then studying the participation of phosphoribosylpyrophosphate in other biochemical reactions, and they alerted me to its role in metabolism. Also, my colleagues at Western Reserve were very interested and supportive, particularly Howard Gest, Robert Greenberg, Abraham Stavitzky, and John Spizizen. We and our families were all good friends and we did everything we could to help one another. Howard Gest, our resident scientist who knew everything, has remained a very close friend; I continually count on him as a reliable source of expert advice and information. With the identification by Frank Gibson of chorismate as the precursor of anthranilate, the intermediates and reactions of the tryptophan pathway were soon identified (Figure 1). Gibson independently contributed to the identification of the intermediates between anthranilate and InGP (11).

While I was at Western Reserve I was visited by Sydney Brenner, at a very early stage in his brilliant career. At the time, Sydney was studying the genes of tryptophan biosynthesis in Salmonella. I also recall an exciting visit by Luigi Gorini, who expounded on his vision of the developing field of bacterial genetics. Harland Wood, chairman of the Biochemistry Department, was the role model for acceptable performance by faculty members at our medical school. He pressed each of us to work very hard, do our very best, and be highly productive. Two graduate students in our department, Oliver Smith and Martin Rachmeler, joined my group, as did Joan Stadler, my first postdoctoral fellow. Oliver and Joan helped with the bacterial studies while Martin continued our investigations with tryptophan desmolase of Neurospora. As our work progressed, I became more and more convinced that *E. coli* would be a more suitable organism than Neurospora for analyzing gene-protein relationships. Because I had little experience performing genetic analyses with bacteria, I decided to spend a summer at the Cold Spring Harbor Laboratory to observe members of the Demerec group who were using transducing bacteriophage P22 in genetic studies with Salmonella. During my stay, Ellis Englesberg and I became very good friends. Ellis subsequently discovered positive control of gene expression in impressive genetic analyses with the *ara* operon of *E. coli*. Upon returning to Western Reserve, I learned that Edward Lennox had shown that transducing phage P1 could be used for genetic analyses with *E. coli*, much like P22 with Salmonella. I contacted Lennox and he and I collaborated in mapping the *E. coli trp* genes; more importantly, our studies

revealed that P1 transduction could be used to construct a fine structure genetic map of any of these genes (12). With this as a possibility, and the increased knowledge about the tryptophan biosynthetic pathway, I again turned to tryptophan desmolase, this time from *E. coli*, as my prime subject for studying the gene-enzyme relationship.

But this decision introduced some serious complications. Genetic and enzymological studies being performed with Joan Stadler and Martin Rachmeler suggested that tryptophan desmolase was more complex than we had imagined. The enzyme appeared to catalyze the last two reactions in tryptophan formation, the conversion of InGP to indole and the condensation of indole and serine to form tryptophan (13). Furthermore, during the enzymatic conversion of InGP to tryptophan, we could not detect free indole as an intermediate (14). What was the significance of this observation? Could the conversion of InGP to tryptophan proceed via a separate reaction without indole serving as an intermediate? Or is the indole that is produced during tryptophan formation retained within the enzyme, and thus undetected? As studies of this enzyme progressed, it developed that this was one of the earliest examples of channeling in an enzyme. The significance of this finding, and its generality, were not fully appreciated at the time, as pointed out by Paul Srere (15). Conclusive proof of indole channeling during catalysis of tryptophan formation by tryptophan synthase (the name of this enzyme changed from desmolase, to synthetase, and finally to synthase) was provided in 1988 by C Hyde, S Ahmed, E Padlan, E Miles, & D Davies (16). They determined the 3-D structure of the four-chain $\alpha\beta_2\alpha$ tryptophan synthase complex for Salmonella, and identified a tunnel connecting the active site of each α subunit with the active site of its adjacent β subunit. Edith Miles, John Dunn, and their coworkers subsequently performed numerous impressive studies establishing the properties of this channel. They also characterized the sophisticated allosteric communication that occurs between the two polypeptides in response to substrate binding at either active site. Edith's analyses with tryptophan synthase strike me as the most impressive enzymological studies I am familiar with. Robert Matthews and Kasper Kirschner have also contributed significantly to our understanding of the properties of this enzyme.

MY MOVE TO STANFORD IN 1958

In 1957 Ed Tatum left Stanford and moved to the Rockefeller Institute in New York. Searching for a young scientist working with Neurospora to replace Tatum, Victor Twitty, chairman of the Biological Sciences Department at Stanford, approached me. He did not realize that most of my research was then based on *E. coli*. At the time I was completing my third year at Western Reserve. Carol and I had just purchased and moved into the first home we owned, in Euclid, a suburb of Cleveland. At the time we had three sons under five years of age. While we were in the midst of painting and furnishing our home, I received the phone call from Twitty inviting me to consider the position at Stanford.

I remember this as a very tough decision. My work was going well and I had enormous respect for my colleagues. Harland Wood, in particular, was inspirational. Because of my admiration for him as school leader, and my friendship for my colleagues, I expressed no interest in considering the invitation from Stanford. But Lester Krampitz, chairman of our Microbiology Department, insisted that I at least visit Stanford. He assured me that he was not hoping that I would leave; rather, he said, he was thinking of the future, and would welcome the opportunity to compete with any outside offer.

I visited Stanford, and frankly, I was not impressed. It was not evident that they would ever catch up with existing outstanding institutions. But the promises made by President Wallace Sterling, Provost Fred Terman, and Dean Phillip Rhinelander seemed sincere to me, and I believed them when they said they were determined to make Stanford the Harvard of the West. CB Van Niel of the Biological Sciences Department Marine Station was assigned the task of wooing me. I recall having lunch with him and I can still visualize the expression of shock on his face when I told him that I did not drink wine. We spent much of the afternoon talking, and he told me why he loved Stanford, and why I should join them. Upon returning to Cleveland, and while pondering this offer, I learned that Arthur Kornberg, Paul Berg, and other members of the Microbiology Department at Washington University Medical School had just decided to move en masse to the Stanford Medical School to become its Biochemistry Department. The Medical School was in the midst of preparing to move from San Francisco to the Stanford campus. I was invited to visit Kornberg's department in St. Louis to meet with and get to know its members, which I did. I stayed with Paul and Mildred Berg, and we became very good friends. This wonderful friendship continues to this day. Arthur Kornberg's reputation, their impending move, the determination of the Stanford administration, and a particularly horrible Cleveland winter convinced me to take the plunge and move. I accepted Stanford's offer, much to the displeasure of my wife, my colleagues at Western Reserve, and my family members in the New York area.

MY PRIMARY FOCUS—ESTABLISHING GENE-PROTEIN COLINEARITY

When I arrived at Stanford in 1958, I was assigned the lab space formerly occupied by the Tatum group, in the basement of Jordan Hall. I thought long and hard about what I should do, and decided that the time had come to mount an all-out attack on the one gene–one enzyme hypothesis. My continuing interest in this problem was stimulated by the problem's redefinition as our understanding of both gene and protein improved. Beadle and Tatum initially proposed the gene-enzyme relationship on the basis of their observation that most mutations that affect a single biochemical reaction could be localized to one specific gene. When this relationship was first suggested, it was not known that a gene is a linear sequence of nucleotides, or that a polypeptide is a linear sequence of amino acids. The findings of Avery,

MacCleod, and McCarty, and Hershey and Chase, and ultimately Watson and Crick in the early 1950s, convinced the scientific community that the DNA double helix serves as the genetic material of most organisms. Benzer's studies in the late 1950s provided support for the view that the linear order of altered sites on a genetic map is representative of the linear order of base changes in a corresponding gene. It was some 20 years later that Maxam and Gilbert introduced the first technique for sequencing DNA. Sanger's studies with insulin, also reported in the early 1950s, established that proteins consist of linear sequences of amino acids.

The first postdoc to join me at Stanford, Irving Crawford, was an exceptionally bright young scientist. Irving, an M.D., had previously worked with Arthur Kornberg for a year, and it was Arthur who persuaded him to join my group as a March of Dimes Postdoctoral Fellow. Arthur, like Harland Wood, is one of those extraordinary individuals whose every word must be taken seriously! Irving's accomplishments in his two years with me were outstanding. It was his findings with *E. coli* tryptophan synthetase that set the stage for our subsequent successful analyses establishing gene-protein colinearity. Irving showed that this enzyme consisted of separable, nonidentical proteins, TrpA and TrpB (17). At the time, this was the first described example of an enzyme composed of nonidentical polypeptide chains. Incidentally, upon completing his stay with me, Irving filled my former position, on the faculty of the Microbiology Department at Western Reserve Medical School.

Several years following Irving's departure, Thomas Creighton characterized this enzyme further. Tom demonstrated convincingly that the enzyme complex catalyzed the last two reactions in tryptophan formation, InGP $\rightarrow$ indole, and indole + serine $\rightarrow$ tryptophan, as well as the overall reaction, InGP + serine $\rightarrow$ tryptophan (Figure 1) (18). In a productive collaboration with Michele Goldberg and Robert Baldwin of our Biochemistry Department, we established that the enzyme is a tetramer consisting of two α chains (TrpA) and one β chain dimer (TrpB) (19). Of particular relevance to our colinearity investigation, Crawford and Creighton demonstrated that each subunit activates the other subunit in the reaction that that subunit catalyzes weakly by itself. It was this property that suggested that we might be able to assay mutant TrpA proteins; although they are inactive in the TrpA reaction, InGP $\rightarrow$ indole, they might activate the TrpB protein in the TrpB reaction, indole + serine $\rightarrow$ tryptophan. Subsequent studies established that many *trpA* mutants do produce an altered TrpA protein and that each does in fact activate TrpB. A second category of *trpA* mutants, those lacking the ability to activate TrpB, were *trpA* nonsense, frameshift, or deletion mutants. Crawford also demonstrated that suppression of one of our *trpA* missense mutants led to the production of an active TrpA protein that was indistinguishable from the wild-type protein (20). The suppressed mutant also formed what appeared to be the parental, mutant protein. These findings supported our hypothesis of a mistake in protein synthesis as the mechanism of suppression.

At this stage in our investigations we knew how to isolate and identify many *trpA* missense mutants, and we could assay and purify each inactive TrpA protein.

We could also map their genetic alterations and use this information to construct a fine structure genetic map of the *trpA* gene. But how were we to determine the amino acid change in each mutant protein? The procedure we ultimately used, peptide fingerprinting, was first described in 1958 by Vernon Ingram. Ingram used this technique to identify the amino acid changes in mutant human hemoglobins. Ingram's two-dimensional fingerprinting procedure separates the peptides in a tryptic or chymotryptic digest. It allows comparison of digests of mutant and wild-type proteins, and identification of any mutant peptide on the basis of its altered mobility. The pure wild-type and mutant peptides could then be isolated and analyzed to determine the amino acid change in the mutant protein. The potential application of this technique to our mutant and wild-type proteins, combined with the procedure we devised for overproduction and purification of the protein, placed us in position finally to be able to test the gene-protein colinearity hypothesis. In 1961 and 1962, Barbara Maling and Donald Helinski characterized several TrpA missense proteins, and Helinski and Ulf Henning applied the fingerprinting procedure to the TrpA proteins of two mutants altered at or near the same site in the *trpA* gene. They excitedly identified different single amino acid changes at the same position in the TrpA protein (21, 22). While these studies were proceeding, Bruce Carlton, John Guest, and Gabriel Drapeau were engaged in the arduous task of sequencing the 268-residue TrpA protein. The sequence took 6 years to complete! When they were done, it was the largest protein to have been sequenced at that time (23). It is amusing that the sequence of amino acids in TrpA, spelled out as a single word, was published as an entry in the 1974 edition of *Mrs Byrne's Dictionary of Unusual, Obscure, and Preposterous Words*!

In our colinearity studies we were aided by other useful procedures. For example, we could isolate numerous *tonB-trpA* deletion mutants (*tonB* mutations confer resistance to phage T1), each with a deletion endpoint somewhere within the *trpA* gene. Such deletions facilitate localization of mutationally altered sites, as Seymour Benzer had demonstrated in his classic studies with the phage T4 rII loci.

By 1964 we had convincingly shown that the order of mutational changes on the genetic map of the *trpA* gene was colinear with the order of amino acid changes in the TrpA protein (24, 25). Our deduced colinear relationship is shown in Figure 2.

Like all hot problems, the establishment of colinearity was a goal of other research groups at the same time. The competing groups had good communication between them, and each group's findings were presented at jointly attended scientific meetings. As often happens in competitive science, Sydney Brenner, with his coworkers Sarabhai , Stretton, and Bolle, established gene-protein colinearity at the same time as we did (26). Most interestingly, though, they used a totally different approach to characterize their mutant proteins. Their analyses were performed with nonsense mutants altered in the phage T4 head protein gene. They compared the sizes of the head protein fragments synthesized by these head protein nonsense mutants; sizes were estimated from proteolytic digests. The estimated polypeptide lengths of the (remaining) head protein fragments of the mutants matched

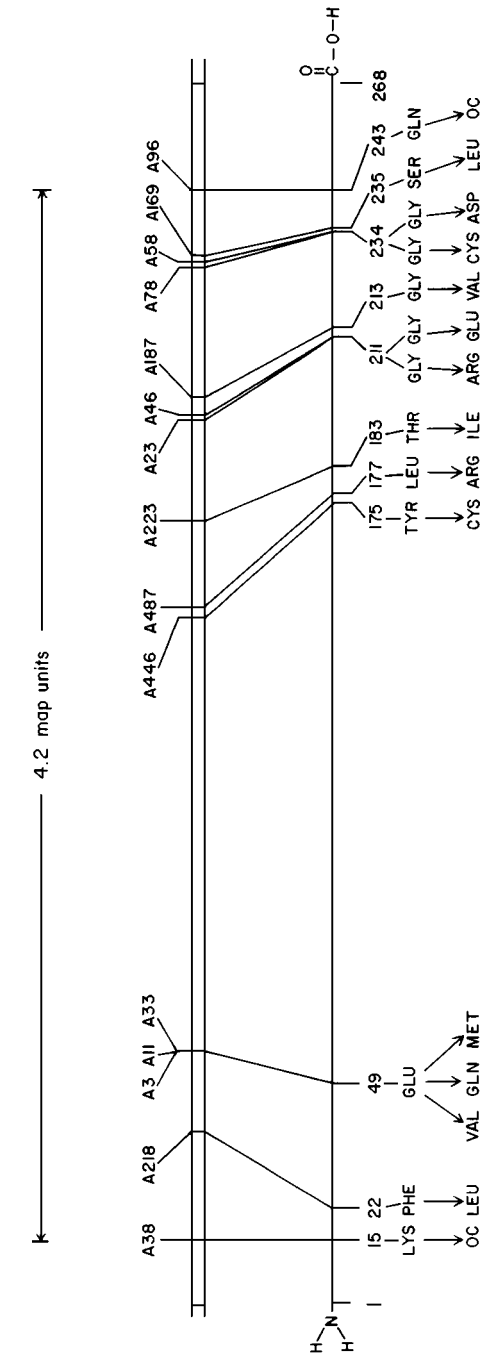

Figure 2 Proof of colinearity of the *trpA* gene and its polypeptide product. The numbers of the *trpA* point mutations examined and the locations of their genetic changes on the genetic map are shown over the schematic gene. The positions of the corresponding amino acid changes are indicated on the linear representation of the TrpA protein.

the order of corresponding nonsense mutations in the head protein gene, proving gene-protein colinearity.

Several other investigators, notably Allan Garen, Frank Rothman, and Cyrus Levinthal, were performing colinearity studies with the alkaline phosphatase of *E. coli*, while William Dreyer and George Streisinger were examining frameshift mutants altered in the head protein of phage T4. Although our findings and those of Sydney Brenner's group were reported first, the findings of these other teams contributed significant basic information on the nature of mutations, and on the translation of the nucleotide sequence of a gene into a protein.

By 1960 it was obvious to everyone working on the colinearity problem that the mutational/genetic/protein strategies we were using should allow us to decipher the genetic code. One additional helpful approach was introduced by Benzer and Ernst Freese, namely, the use of chemical mutagens to produce specific base changes in DNA. Using this refinement, it was likely that we could correlate numerous specific base changes in *trpA* with specific amino acid changes in the TrpA protein, and thereby decipher the genetic code (27). While their studies were under way we learned of Nirenberg's spectacular in vitro experiments that were destined to reveal the complete genetic code. Despite missing out on our primary objective, we did demonstrate genetic recombination within a codon (28). We also characterized many specific amino acid changes in TrpA that were consistent with codon predictions in vitro, and with the assumption that single mutational events are generally restricted to a change in a single base pair. John Guest, William Brammar, and Hillard Berger of my group participated in these studies. In related studies, Edward Cox examined *E. coli* strains containing the *mutT* mutator gene (our findings suggested that *mutT* causes AT to CG transversions) and demonstrated that the presence of this mutant locus led to an overall change in genome base composition during continuous growth of this bacterium (29). This observation suggested that the bacterial genome can tolerate numerous, presumably inconsequential, nucleotide changes. The significance of this observation was pointed out in the writings of Tom Jukes (cited in 30).

In other, related studies, Donald Helinski discovered what we called second site reversion—a mutation introducing a second amino acid change in a TrpA mutant protein, which restores enzymatic activity (31). Some years later, when the 3-D structure of tryptophan synthase of Salmonella was solved, by Hyde et al (16), it became evident how these second site amino acid changes acted.

During the late 1950s, the demands on my time continued to increase and it became apparent that I could no longer spend much of the day at the lab bench. I decided to employ two research assistants, one to perform genetic procedures under my supervision, and a second to carry out the biochemical analyses I wished I could perform with my own hands. Virginia Horn joined my group in 1958 and worked with me until she retired in 2000. She is partly responsible for many of the genetic contributions from my lab. For my biochemical studies I had a stream of talented assistants, including Donald Vinicor, Jun Ito, Deanna Thorpe, Susan Stasiowski, Joan Hanlon, Magda van Cleemput, Jan Paluh, and M-C Yee. The

participation of these coworkers in our lab's activities kept me engaged at a level that would not have been possible if my interactions were solely with students and postdocs.

CLARIFYING MISSENSE SUPPRESSION

In the early 1960s, it was apparent that the approaches we were using in our colinearity studies could be applied to explain missense suppression. Stuart Brody undertook this project. He used peptide fingerprinting and amino acid sequence analyses, with purified wild-type, mutant, and suppressed mutant TrpA proteins, to show that his suppressed mutants produce two TrpA proteins, one that is indistinguishable from the parental mutant protein, and the second identical to the wild-type protein (32). 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. He considered other possibilities as well. Peggy Lieb and Len Herzenberg also proposed that mutant tRNAs may be responsible for suppression.

In the early 1960s, Paul Berg and I began an avid tennis competition at Stanford that had a bearing on the mechanism of missense suppression. Between games, we enjoyed sitting on the bench, discussing our respective research programs. We occasionally dreamt up clever experiments that had never been attempted. On one such occasion, I must have been discussing missense suppression, and I asked Paul how we could prove that it was due to an altered tRNA? At that time it had already been established that nonsense suppression was caused by an altered tRNA. Paul concocted a very pretty experiment that could be performed with the tRNA from our missense suppressor strains, but it depended on having template RNAs with defined sequences. He was certain that Gobind Khorana could prepare DNAs that could be used to produce RNAs with just the sequences we wanted. The idea was so attractive to Paul, and to Gobind, that Paul convinced John Carbon, a visitor to his lab, to perform the experiment with one missense suppressor system, while Khorana and his coworkers undertook a parallel analysis with a second missense suppressor. The objective was to show that bulk tRNA from a strain with a missense suppressor would mistranslate a specific mutant codon in a synthetic RNA, and introduce the "incorrect" amino acid into the peptide product. The results were beautiful; they demonstrated the expected mistranslation with the tRNAs of the two suppressor mutants (33, 34). Subsequently, other investigators, most notably Michael Yarus, who had studied with Paul, identified many of the nucleotide changes in these and other suppressor tRNAs.

My interest in suppression and its many ramifications continued into the early 1970s. Emanuel Murgola exploited forward and reverse mutational changes, and suppression, to introduce many amino acid changes at specific positions in TrpA (35). His findings suggested that there was much yet to learn about alternative mechanisms of missense suppression. I concluded, correctly, that I could leave the

suppression problem because it was in good hands. Murgola has since discovered numerous features that were not previously appreciated, including the observation that altered ribosomal components affect the specificity of decoding.

trp mRNA DEGRADATION AND OTHER STUDIES

In the early 1960s, A Matsushiro described a lambda-like phage, ϕ80, that has a chromosomal integration site just beyond *trpA* of the *trp* operon. Upon excision, adjacent segments of the *trp* operon are removed with the phage genome, generating transducing phage. These phage proved to be invaluable, for their DNA could be used to measure mRNA levels corresponding to any gene in the *trp* operon, by RNA:DNA hybridization. This phage was also used in an important unrelated study by Naomi Franklin, while she was in my lab, performed with William Dove (36). They integrated the genome of a *trp* transducing phage into the chromosome of *E. coli,* and used deletion mapping to establish that the phage genome was linearly inserted, supporting the Campbell integration model.

While we were isolating *trpA* missense mutants, we also recovered many nonsense and frameshift mutants altered in the various genes of the *trp* operon. Many of these mutants, especially those altered near the beginning of a gene, had so-called polar, negative effects on synthesis of Trp polypeptides specified by the genes downstream in the operon. Comparable observations had been made previously by investigators studying many operons. Searching for an explanation for polarity, we used the DNA of a set of *trp* transducing phage to measure *trp* mRNA levels corresponding to the different *trp* genes, and discovered that the distal *trp* mRNA levels were very low, presumably due to transcription termination (37). A few years later Rho factor was discovered by Jeffrey Roberts, and it was shown that Rho factor was responsible for the premature transcription termination associated with polarity. Rho protein binds to untranslated segments of a mRNA and searches 3′ for a paused polymerase. If it encounters one, transcription is terminated. The stabilities of different segments of *trp* mRNA were determined in *trp* polar mutants, and it was observed that the untranslated mRNA segment just distal to the introduced "polar" stop codon was exceptionally labile (38). Apparently following polarity-associated Rho-dependent termination, the unnatural 3′ end of the prematurely terminated transcript is subjected to rapid 3′ to 5′ degradation.

By the late 1960s, it had become apparent from our mRNA measurements that *trp* mRNA levels accurately reflected the levels of the *trp* operon-encoded polypeptides. Armed with pure DNA from ϕ80 phage bearing different desired segments of the *trp* operon, we used RNA:DNA hybridization to follow *trp* mRNA synthesis and decay. This was essential to do, to verify that the operon was a single transcriptional unit that specified a transcript 6000+ nucleotides in length. This expectation was verified, and it was also shown that transcription of the operon, translation of its nascent transcript, and degradation of the transcript all proceed simultaneously (39, 40). Our general conclusions were verified visually by elegant electron microscopic studies performed by Sarah French et al (41).

They photographed transcription and translation in progress on the *trp* operon in strains bearing plasmids containing the intact operon of Salmonella, or the operon with segmental deletions.

At this time we were also interested in determining the direction of *trp* mRNA decay. Our initial study focused on *trp A*mRNA, at the 3′ end of *trp* operon mRNA, and suggested that degradation proceeds in the 3′ to 5′ direction (42). Subsequently, decay of the complete *trp* transcript was analyzed, and we discovered that most *trp* mRNA molecules were undergoing degradation in their 5′ segments, as their synthesis was proceeding. We considered this result to be contradictory to our earlier findings, and in fact published a paper in *Nature* entitled "Direction of in vivo degradation of tryptophan messenger RNA—a correction" (43). We now know that both results were correct. Attack on most *trp* mRNAs is initially endonucleolytic, and occurs in the 5′ segment of *trp* mRNA. Each *trp* mRNA fragment that is generated then is probably degraded exonucleolytically, in the 3′ to 5′ direction. Only about 50% of the *trp* mRNAs remain full-length as they are being synthesized. During these studies it became apparent that the enzymology of decay was complex, and I therefore dropped this project. Our mRNA decay studies were performed by Robert Baker, Daniel Morse, Raymond Mosteller, Fumio Imamoto, Jun Ito, Jes Forchhammer, Ethel Jackson, Jack Rose, and Barry Marrs.

In other studies, John Hardman attempted to identify residues in the TrpA protein that are catalytically essential (44). David Jackson performed some elegant denaturation/renaturation experiments with mixtures of inactive mutant TrpA polypeptides that are normally monomeric, which yielded functional mixed dimers (45, 46). James Spudich attempted to determine the explanation for the different classes of *tonB-trp* operon deletion mutants that are isolated in *E. coli* strain B, vs K-12 (47).

During the early 1970s, recombinant DNA analyses were beginning to blossom. Donald Helinski, at the University of California at San Diego, recognized that the plasmid he was studying, colE1, had many characteristics that would be ideal for recombinant DNA investigations. His studies reached the stage where he wished to introduce a well-studied operon, such as the *trp* operon, into his plasmid, and demonstrate expression amplification. Herbert Boyer and I helped Vickers Herschfield and Michael Lovett of the Helinski group with this project as they successfully demonstrated expression amplification of the *trp* operon (48). Many labs promptly adopted colE1 and its derivatives in their recombinant DNA research.

REGULATION OF OPERON EXPRESSION BECOMES OUR MAJOR INTEREST: DISCOVERY OF TRANSCRIPTION ATTENUATION

My group's concern with gene-protein colinearity, the mechanism of suppression, and the genetic code largely ended in the mid-1960s. Our focus shifted as we set out to analyze how the *trp* operon was regulated. In 1959, Georges

Cohen and François Jacob had identified a regulatory locus, *trpR*, which when mutated, conferred bacterial resistance to tryptophan analogs. They postulated, correctly, that *trpR* encodes a repressor protein, analogous to the *lac* repressor, that responds to the accumulation of tryptophan by down-regulating *trp* operon expression.

We verified that *trpR* does in fact specify a protein, identified the *trp* operon transcription start site, and set up an in vitro transcription system for the purpose of analyzing *trp* repressor action. These experiments were performed by Catherine Squires and Jack Rose of my group, in 1973, with the expert help of HL Yang and Geoffrey Zubay of Columbia University. Partially purified *trp* repressor was shown to be activated by L-tryptophan and, when active, it repressed transcription initiation (49). During this period we were well aware of the comparable regulatory studies being conducted by Bruce Ames and his group with the histidine operon of Salmonella. Their findings implicated tRNAHis, not free histidine, as the signal molecule that is sensed in *his* operon regulation. We took their findings very seriously, since both histidine and tryptophan are minor amino acids. Ford Doolittle of my group in fact performed experiments designed expressly to examine the potential role of tryptophanyl-tRNA synthetase function in repression of *trp* operon transcription. He concluded, correctly, that neither tryptophanyl-tRNA synthetase nor tRNATrp participates in repression of *trp* operon expression (50). Only years later could we explain the observation that some of his mutants exhibited increased *trp* operon expression; this increase was due to relief of attenuation.

In our regulatory studies on *trp* operon expression, we were surprised to find that mutants lacking a *trp* repressor still responded to tryptophan starvation by increasing their rate of *trp*mRNA synthesis (51). Did this imply that a second tryptophan-responsive regulatory mechanism controls transcription of the *trp* operon? This question was emphatically answered "yes" by Ethel Jackson of my group. At the time, Ethel was attempting to locate the *trp* operon's internal promoter. Among the deletions she isolated were two that increased transcription of the operon three- to sixfold. These deletions had one endpoint just following the promoter/operator region and the second near the end of *trpD*, the second structural gene in the operon (52). This increase occurred regardless of whether the cell could or could not form a functional *trp* repressor. At about the same time, a similar observation was reported by T Kasai based on his studies with the *his* operon of Salmonella; deletions removing the initial portion of the *his* operon also increased operon expression (53). Both Jackson and Kasai postulated, correctly, that a regulated site of transcription termination must exist in the initially transcribed regions of these operons, and that deletion of this site increases operon expression. Kasai introduced the term attenuation (regulated downstream gene expression) to describe this regulatory mechanism. As this term was entirely appropriate, we and others adopted it.

Subsequent studies by Fumio Imamoto, then back in Japan, confirmed by Sota Hiraga of my group, revealed that transcription in progress in the leader region of the *trp* operon could be stopped abruptly by providing tryptophan to a

tryptophan-starved culture (54, 55). This finding and Ethel Jackson's observation, both suggesting the existence of a site of transcription termination, were solidified experimentally by the analyses of Kevin Bertrand and other members of my group (56, 57). The distinct site of transcription termination was located just before *trpE*, in the distal portion of the *trp* operon leader region. Most importantly, it was discovered that the decision whether or not to terminate transcription at this site was based on the cellular level of charged tRNATrp, not tryptophan. With these results in hand, we were finally beginning to see the similarities between transcription regulation in the *trp* and *his* operons.

The years immediately following were an extraordinarily exciting period for my group. What was most remarkable was that each member thought he or she was principally examining a separate process influencing *trp* operon expression. As it turned out, each was analyzing a different aspect of the same process, transcription attenuation. Craig and Catherine Squires, Morley Bronson, Frank Lee, Kevin Bertrand, and Philip Cohen of my group, with the help of Moshe Yaniv, used elaborate RNA sequencing procedures to determine the complete sequence of the 162-base-pair transcribed leader region that precedes *trpE*, the first major structural gene of the operon (58). While these sequencing studies were under way, Maxam-Gilbert DNA sequencing technology became available, and it was used to confirm the deduced DNA sequence. Separately, Frank Lee was examining *trp* leader RNA, searching for possible RNA secondary structures. It was Lee who predicted, and then experimentally demonstrated, that alternative RNA hairpin secondary structures are responsible for the antitermination and termination events (59). In other studies, Terry Platt and Craig Squires discovered an unsuspected ribosome binding site and peptide coding region in the leader transcript (60). Particularly exciting was the realization that this coding region would specify a 14-residue leader peptide containing tandem Trp residues. The corresponding Trp codons qualified as the potential sites at which the availability of charged tRNATrp could be sensed, thus providing a possible explanation of how charged and uncharged tRNATrp could regulate *trp* operon expression. Gerard Zurawski, Dirk Elseviers, and George Stauffer, with Dale Oxender, a frequent sabbatical visitor, aided by Larry Soll of the University of Colorado, a former graduate student of Paul Berg, then demonstrated that the inability to translate the Trp codons in the leader peptide coding region was the key event that led to the formation of Frank Lee's antiterminator RNA structure (61–63). Independently, Daniel Morse and his coworkers established the role of tRNATrp charging in *trp* operon regulation by attenuation. By 1977 we understood many of the basic molecular features of this form of transcription attenuation.

Because my goal was to understand the physiological behavior of the organism, I could not ignore the importance of feedback inhibition of enzyme activity. Therefore we isolated and analyzed several feedback resistant mutants and showed that these were all altered in *trpE*, the gene specifying one polypeptide of the enzyme complex that catalyzes the first two reactions in tryptophan formation (see Figure 4, discussed in more detail below). We purified the anthranilate synthase complex

and demonstrated that tryptophan feedback inhibits both reactions. However, the separated TrpGD polypeptide was not subject to feedback inhibition. Jun Ito and Edward Cox performed these initial analyses (64, 65). More thorough studies on this enzyme complex, from Salmonella and other organisms, have been carried out by Howard Zalkin and Ron Bauerle and their coworkers and collaborators.

INVESTIGATING REPRESSION

By the late 1970s, it was apparent that both repression and transcription attenuation regulate *trp* operon expression. However, we did not understand either process well enough to assess the relative importance of these regulatory mechanisms. Repression appeared to be more starvation-sensitive than attenuation, but we had not thoroughly examined this observation. I decided to devote more attention to repression. We located the operator more precisely by isolating several operator-constitutive mutants and determining their sequence changes. We also carried out in vitro repressor-operator binding and protection studies. George Bennett, Ernst Schweingruber, Craig Squires, Daniel Oppenheim, and visiting scientist Keith Brown participated in these investigations (66, 67). Robert Gunsalus took over from this group and, with Gerard Zurawski, cloned *trpR* and subsequently sequenced it (68, 69). *trpR* also was cloned and sequenced by my former postdoc, Ronald Somerville, and his group (70). Gunsalus identified homologous operator sequences in two additional *trp* repressor-regulated operons, *trpR* and *aroH* (69). Subsequently, *mtr* and *aroL*, two other operons concerned with aromatic amino acid metabolism, were shown to have *trp* repressor–regulated operators.

Structure/function/mechanism-of-action studies were initiated with the *trp* repressor by Richard Kelley of my group, who concluded that the repressor was probably a helix-turn-helix DNA binding protein (71). In the early 1980s, Paul Sigler, then at the University of Chicago, became interested in the *trp* repressor and adopted it as his preferred protein for X-ray crystallographic analysis. Oleg Jardetsky of Stanford also set out to determine the structure of this protein, using nuclear magnetic resonance technology. We were hopeful that one or both would succeed, so that we could use their structural data to interpret repressor action. Both groups were successful, and the structures of the aporepressor, repressor, and repressor-operator complex were determined. These structures explain how tryptophan activates the aporepressor, and how repressor recognizes its target operator. An unexpected observation was that water molecules appear to mediate contacts between repressor and operator. Lisa Klig and Barry Hurlburt of my group, with Tom Graddis and Dale Oxender at the University of Michigan, and independently Janette Carey of Princeton, identified many additional features of the *trp* repressor (72, 73). After leaving my lab, Robert Gunsalus contributed the interesting finding that there appear to be multiple repressor binding sites in the *trp* operon operator region (74). Catherine Lawson and Janette Carey provided conclusive structural evidence in support of this proposition (75).

CONTINUING STUDIES ON ATTENUATION

In the mid- to late-1980s, we were determined to explain all the structural and functional features of transcription attenuation. In particular, we wanted to know how transcription and translation were coupled in the leader region of the operon. During this period Malcolm Winkler of my group was studying transcription of the *trp* operon leader region, in vitro, using single-round transcription analyses (76). In this procedure rifampicin is added soon after transcription begins, so that only one round of polymerase molecules is allowed to transcribe. Their movement on the DNA template was then followed by labeling and isolating their RNA products. Using this method, we discovered a previously undetected major transcription pause site—the paused RNA species corresponds to the most 5′of the three alternative hairpin structures that can form in the *trp* leader transcript. This pause event was analyzed further, although initially we did not appreciate its importance (76, 77). Robert Fisher, Anath Das, and Roberto Kolter also participated in these studies.

Winkler also performed some pretty experiments with the help of Cary Mullis of the Cetus Corporation. Mullis synthesized oligonucleotides, or oligos, for us that were complementary to the different strands of the leader RNA hairpin structures that we believed were responsible for termination and antitermination. This was at a time when DNA synthesis technology was not generally available. These oligos were used in vitro to establish the roles of the leader RNA secondary structures in antitermination and termination. Each added oligo presumably paired with its complementary RNA sequence, disrupting the corresponding hairpin structure and eliminating its function (78). Independently, Iwona Stroynowski prepared overlapping deletions that removed sequential segments of the *trp* operon leader region of *Serratia marcescens* and confirmed the importance of the three leader RNA secondary structures, and leader peptide synthesis, in regulation by attenuation (79). To verify the crucial role of RNA polymerase in the attenuation process, we isolated rifampicin-resistant mutants that exhibited either relaxed or more stringent attenuation regulation. These mutants were altered in *rpoB*, the structural gene for the polymerase beta subunit. The altered behavior of these polymerases was demonstrated in vitro (80). These mutants continue to be widely used in studies on polymerase action.

Despite the many human-years of labor devoted to analyzing transcription attenuation in the *trp* operon, several basic features could not be explained. Most importantly, we did not understand how translation of the leader transcript was coupled with transcription of the leader region, which it must be if translation is to regulate transcription termination. We also did not know how basal level expression of the operon, i.e. expression in the presence of adequate levels of tryptophan, was set. These features were explained by Robert Landick, Janette Carey, and James Roesser with the help of Yoshikazu Nakamura of Tokyo University. Landick and Carey showed that the early pause structure identified by Winkler is in fact responsible for the coupling of transcription with translation. The polymerase molecule that has synthesized this pause structure is released from the pause complex by the ribosome synthesizing the *trp* leader peptide. Then this polymerase

and ribosome move in unison on their respective template and transcript (81). Depending on whether there is sufficient charged tRNATrp to allow the translating ribosome to translate the two leader Trp codons, the antiterminator or terminator structure forms, regulating downstream transcription. Basal level expression was shown to be influenced by the relative stabilities of the leader RNA secondary structures, the rate of leader peptide synthesis, and the rate of ribosome release (82). Figure 3 presents an overview of the stages in *trp* operon attenuation.

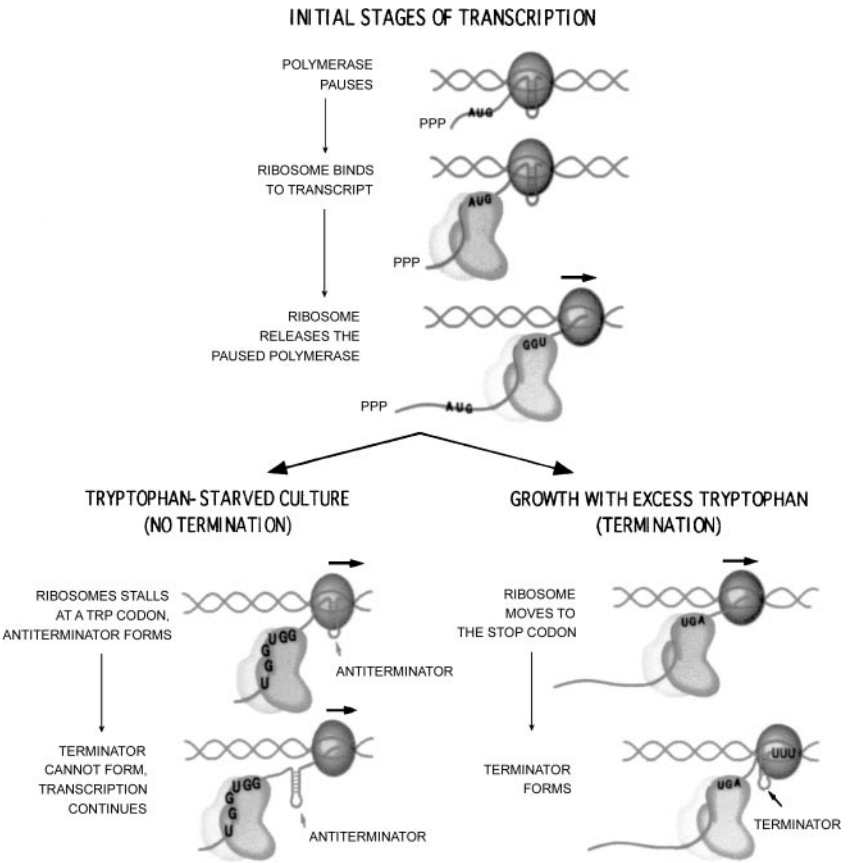

Figure 3 The major stages in transcription attenuation regulation of the *trp* operon of *E. coli*. Stage 1: transcription pausing and ribosome release of the transcription pause complex. Option 1 (*left*): cultures deficient in charged tRNATrp. The ribosome translating the leader peptide coding region stalls at one of the Trp codons; this allows the RNA antiterminator structure to form. The antiterminator prevents formation of the terminator, permitting transcription to continue into the structural gene region of the operon. Option 2 (*right*): cultures growing with adequate levels of charged tRNATrp. The RNA terminator structure forms, causing transcription termination.

Because tRNATrp charging is central to attenuation regulation of *trp* operon expression, it was essential that we learn whether *trpS*, the structural gene for tryptophanyl tRNA synthetase, was regulated by tryptophan or tRNATrp. Carol Hall cloned, sequenced, and analyzed expression of *trpS* and showed that it is not regulated by either tryptophan or tRNATrp (83). Expression of *trpS* of *E. coli* is growth-rate regulated, but how, we don't know.

The information gathered in our studies on repression and attenuation in the *trp* operon of *E. coli* provided a logical explanation for the existence of these two different regulatory mechanisms. Repression, responding to the availability of free tryptophan, allows about an 80-fold range in operon expression. Attenuation, responding to the accumulation of uncharged tRNATrp, permits about a sixfold range of expression. Thus, when cells are severely starved of tryptophan, and lack charged tRNATrp, transcription of the structural gene region of the *trp* operon could be increased almost 500-fold. However, because relatively low levels of charged tRNATrp are sufficient to sustain appreciable protein synthesis, termination is not relieved at all until repression is practically completely relieved. Thus, by using two regulatory mechanisms that sense different molecules, *E. coli* can fine-tune tryptophan biosynthesis to sustain adequate protein synthesis.

As our understanding of the features of repression and attenuation in the *trp* operon of *E. coli* improved, we became concerned with the question, how do other bacterial species regulate expression of their *trp* operons? Are the mechanisms similar, or are they different? Sequence comparisons revealed that the *trp* operon regulatory regions of many bacterial species have features analogous to those of the *trp* operon of *E. coli*; thus they are probably regulated similarly. However, in some species the *trp* genes are organized differently, and unrelated regulatory strategies are evident. (See the section below on regulation of the *trp* operon of *Bacillus subtilis*.) These comparative studies were performed by Frank Lee, Kevin Bertrand, Brian Nichols, Iwona Stroynowski, Miroslav Blumenberg, Giuseppe Miozzari, George Bennett, and Michael Manson.

Like most investigators studying homologous proteins from different species, I became interested in the significance of the many amino acid differences that are observed. To address this question using a *trp* operon protein, in 1980–1981 William Schneider and Brian Nichols produced recombinants, in vivo, between *trpA* of *E. coli* and *trpA* of *Salmonella typhimurium*. The TrpA proteins of these species have 40 differences in their 268 residues. Although we did not select for TrpA enzymatic activity, all recombinant TrpA proteins were found to be indistinguishable catalytically from the parental TrpA proteins (84). This result suggests that the 40 amino acid differences in these homologous proteins are largely inconsequential, catalytically.

Translational Coupling

By 1981 the entire *trp* operon had been sequenced (85). The organization of the operon, associations of its products, and the reactions the enzymes catalyze are

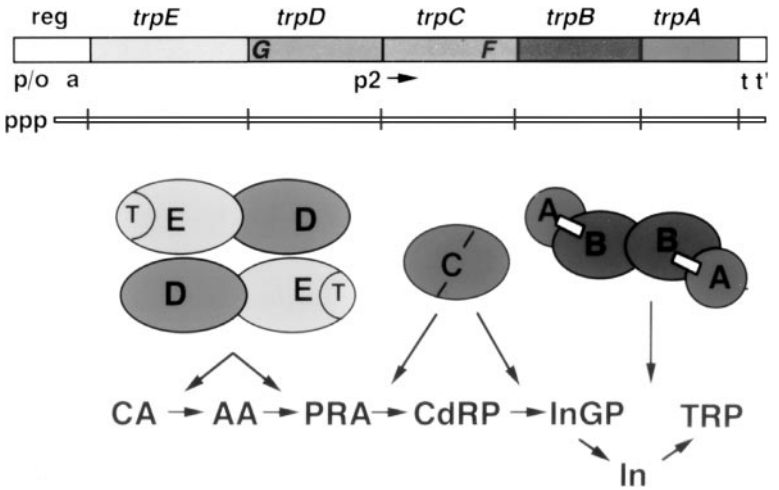

Figure 4 Organization of the genes and enzymes of the *trp* operon of *E. coli*. The locations of the promoter/operator (p/o), attenuator (a), internal promoter (p2), and tandem terminators (tt′) are indicated. The various enzyme complexes formed from the five Trp polypeptides are shown. The tryptophan feedback inhibition site in TrpE is represented by T. Tunnels are shown connecting the active sites of TrpA and TrpB. The reactions catalyzed by the Trp enzymes are indicated; see Figure 1 for the reactions.

summarized in Figure 4. The sequence revealed several interesting features. For example, the coding regions for two pairs of adjacent *trp* genes, *trpE-trpD* and *trpB-trpA*, have overlapping stop and start codons, UGAUG, and the polypeptides specified by each gene pair form an enzyme complex. Coexpression of these adjacent genes was examined and it was observed that their translation was coupled, i.e. whenever translation of the *trpE* or *trpB* coding region was prematurely terminated by introducing a stop codon, expression of the downstream coding region, *trpD* or *trpA*, was markedly reduced (86). This was not due solely to Rho-mediated transcription termination. Rather, the ribosome binding site and start codon segment for each downstream gene is so designed that initiation is partly dependent on translation proceeding to the stop codon of the upstream coding region. *trpB-trpA* translational coupling was analyzed in some detail, and it was shown that transcript secondary structure, ribosome binding site efficiency, and possibly other sequence features influence downstream gene expression (87). Inspection of the sequences of many bacterial genomes has revealed the existence of numerous overlapping coding regions. These are potential sites of some form of translational coupling. The *trp* operon was sequenced as a collaborative effort by members of my group, and by Irving Crawford, Terry Platt, and their coworkers. Translational coupling was studied by Daniel Oppenheim and Anath Das.

NEUROSPORA REVISITED

In the late 1970s Eric Selker joined my group as a graduate student. His undergraduate studies were at Reed College, where he worked on a Neurospora project under the guidance of my close friend Gabe Lester. In his first two years with me, Selker examined the properties of the ribosome binding sites of several of the *trp* genes of Salmonella. I was unaware when he joined my group that he was determined to perform his thesis research using Neurospora. When he first raised this possibility I am sure I argued against it, but Selker obviously prevailed. I did not realize that by permitting him to work with Neurospora I would be opening the door to many prospective eukaryote biologists. Within a few years I was forced to limit the size of my Neurospora group; otherwise my bacterial research would have been terminated!

When Selker initiated his Neurospora studies with me, very little molecular technology had been developed for use with this organism. Yeast and Drosophila were the lower eukaryotes of choice for molecular genetic investigations. We were well aware of this deficiency, and Selker selected what we thought would be a straightforward, feasible project—characterization of the 5S ribosomal RNA genes of Neurospora. Rather quickly it became apparent that unlike most organisms, in which 5S genes are clustered, in Neurospora the 100 or so 5S genes are dispersed throughout the genome (88). Selker uncovered important differences between these separate genes, and continued the most interesting aspects of this problem as a postdoc with Robert Metzenberg.

It was evident from Selker's experience that we lacked the tools needed to perform up-to-date molecular studies with Neurospora. Therefore, the graduate students and postdocs who came to my lab to work with Neurospora devoted some of their time to technology improvement. Steven Vollmer increased the efficiency of DNA transformation with Neurospora, and prepared the first ordered cosmid libraries (89). These were used by many investigators to clone their favorite Neurospora genes. Vivian Berlin initiated analyses of asexual spore formation (conidiation), and devised a procedure for synchronizing the stages in this developmental process. She also prepared cDNAs from the mRNAs that are predominantly present during sporulation and used them to clone a set of so-called *con* genes that are transcriptionally active principally during conidiation (90). We used these genes to monitor the effects of development, light, and circadian rhythm on *con* gene expression, and to identify one major regulatory gene, *rco*-1, concerned with the conidiation pathway. Marc Orbach collaborated with Douglas Vollrath and Ronald Davis of our Biochemistry Department in applying Gilbert Chu's alternating-field gel electrophoresis technique to separate the seven chromosomal DNAs of Neurospora (91). Others in my lab analyzed genes concerned with carotenoid and chitin biosynthesis. The protein product of the gene *cpc*-1, a homolog of the well-studied yeast protein GCN4, like GCN4, was shown to be subject to translational regulation. One additional successful project involved cloning and analysis of the

nonhomologous mating-type regions of Neurospora. The latter studies were performed by Chuck Staben in collaboration with Louise Glass and Bob Metzenberg (92). The individuals who worked with Neurospora in my lab include those mentioned above plus Daniel Ebbole, Michael Plamann, Matthew Sachs, Oded Yarden, Thomas Schmidhauser, Frank Lauter, Matthew Springer, Luis Corrochano, Carl Yamashiro, Bheong-Uk Lee, Dennis Burns, Brian White, Anne Roberts, Jan Paluh, Tim Legerton, Michael Schechtman, Karl Hager, and others. In addition, my good friends Irving Crawford and Howard Zalkin spent some leave time with me, helping us with some risky related projects. My greatest personal reward from conducting these studies was seeing so many talented young investigators develop careers in science exploiting Neurospora or a related organism.

CONTINUING BACTERIAL STUDIES

Attenuation Regulation of the *trp* Genes of *Bacillus subtilis*

After Brian Nichols and others in my group initiated comparative studies on *trp* operon organization and regulation in different species, *B. subtilis* stood out as an attractive subject for further investigation. Earlier work had established that *B. subtilis* has seven *trp* genes encoding the seven protein domains required for tryptophan synthesis, and that these genes are regulated by the gene designated *mtrB*. Six of the *trp* genes are organized as a *trp* operon and the seventh, *trpG*, is in a folate operon. The location of *trpG* is logical, for the TrpG polypeptide of this organism is a component of two enzyme complexes, one that participates in tryptophan formation and the other that functions in folate synthesis. Our initial regulatory studies with *B. subtilis* were performed by Mitzi Kuroda; her objective was to sequence and characterize the regulatory region of its *trp* operon. While her analyses were under way I learned that Dennis Henner of nearby Genentech also was studying the *trp* genes of this organism. We joined forces with Henner, who was aided by H Shimotsu, a postdoc in his group. The nucleotide sequences of the *trp* operon leader regions of *B. subtilis* and of the related organism *B. pumilus* suggested that their leader transcripts should be able to fold to form alternative antiterminator and terminator structures (93). However, neither leader sequence contained a peptide coding region; therefore it was not obvious how tryptophan or tRNATrp could be sensed as a regulatory signal. An additional complicating feature was the location of the *B. subtilis* trp operon within a supraoperon that contains six additional genes, three upstream and three downstream of the six *trp* genes (94). These six genes encode enzymes catalyzing reactions of the common aromatic pathway, and of phenylalanine and tyrosine biosynthesis.

In our initial regulatory studies with the *trp* operon, the most tantalizing finding was that overproduction of a specific segment of the *trp* leader transcript in *trans*, in vivo, relieved transcription termination in the *trp* leader region. This implied

that regulation might be mediated by a regulatory protein that acts by binding to a specific segment of the transcript. We presumed that this protein was the product of the regulatory gene, *mtrB*. Paul Gollnick and Shuichi Ishino of my group joined with Kuroda and Henner in demonstrating that *mtrB* does in fact encode a tryptophan-activated RNA-binding regulatory protein, and that this protein does regulate transcription termination in the leader region of the *trp* operon (95). Subsequently, Paul Babitzke of my group purified MtrB (renamed TRAP, for *trp* RNA-binding attenuation protein) and showed that its RNA binding site overlaps the antiterminator, and consists of repeated (A/G)AG sequences (96). We now know that TRAP wraps the antiterminator sequence around its periphery, disrupting its structure. This frees nucleotides at its base that form part of the terminator, which causes termination. Donald Staley and Irving Crawford identified a TRAP-binding sequence that overlaps the *trpG* ribosome binding site. After leaving my lab, Gollnick and Babitzke showed that TRAP does bind and inhibit *trpG* translation. The continuing contributions of Babitzke and Gollnick on this project have solidified our understanding of TRAP action. Gollnick's group has collaborated with Alfred Antson and coworkers in solving the 3-D structures of tryptophan-activated TRAP, and of activated TRAP complexed with a target RNA (97, 98). These beautiful structures revealed the details of how TRAP functions.

Other studies, performed by Enrique Merino and by the Babitzke group, confirmed a prediction by Mitzi Kuroda that TRAP binding to *trp* leader RNA would promote formation of an additional leader RNA hairpin structure, one that would block the *trpE* ribosome binding site (99). Studies by Joseph Sarsero, performed with undergraduate Alfred Lee, confirmed an earlier observation by others that both the *trp* operon and *trpG* are up-regulated in response to the accumulation of uncharged tRNATrp (100). The operon directly regulated by uncharged tRNATrp was identified; it contains genes of unknown function, *yczA* and *ycbK* (101). Expression of this operon prevents TRAP action. These studies, by Sarsero and Merino, now back in Mexico, are being continued by Angela Valbuzzi of my group. Perhaps the two most interesting findings with the *trp* operon of *B. subtilis* are that its attenuation mechanism is totally unlike the one regulating *trp* operon expression in *E. coli*, but as in *E. coli*, both tryptophan and tRNATrp are sensed as regulatory signals.

Attenuation Regulation of the Tryptophan Degradative Operon

E. coli can also degrade tryptophan. Breakdown is catalyzed by the enzyme tryptophanase, one of two polypeptides specified by the two-gene tryptophanase (*tna*) operon. The operator proximal gene, *tnaA*, encodes the degradative enzyme, whereas *tnaB* specifies a tryptophan-specific permease. Our studies with this operon were initiated by Michael Deeley in the early 1980s. When he began his

studies it was known that expression of the *tna* operon was subject to catabolite repression and was induced by tryptophan. The operon was cloned and sequenced, and it was confirmed that initiation was regulated by catabolite repression (102). Valley Stewart continued these investigations and showed that the 300+base-pair leader region had regulated sites of Rho-dependent transcription termination that function except when cells have high concentrations of tryptophan. Stewart established the participation of the 24-residue tryptophan-containing leader peptide, TnaC, in tryptophan-induced transcription antitermination (103). His work, and that of Bob Landick, Paul Gollnick, Ajith Kamath, Vincent Konan, and Kurt Gish, have defined many of the features of this mechanism of attenuation (104). Yoshikazu Nakamura of Tokyo University also provided aid.

Currently, Feng Gong is exploiting an in vitro system to analyze the specific events of induction. The findings of Vincent Konan, Ajith Kamath, and Kurt Gish suggested that tryptophan induction is a consequence of inhibition of ribosome release at the *tnaC* stop codon; the stalled ribosome presumably blocks transcript sites required for Rho binding or action. Consistent with this interpretation, Feng Gong has recently observed that induction leads to the accumulation of TnaC-peptidyl-tRNA. The peptidyl-tRNA probably prevents its associated ribosome from releasing at the leader peptide stop codon, thereby blocking Rho action. These studies are continuing.

In addition to the three attenuation mechanisms I have described, each regulating some aspect of tryptophan metabolism, a fourth attenuation mechanism, responding to uncharged tRNATrp, also influences tryptophan metabolism in *B. subtilis*. This T box mechanism regulates expression of many operons, including the operon containing *trpS*, the structural gene for tryptophanyl-tRNA synthetase (105). T box control was discovered by Frank Grundy & Tina Henkin (105). This mechanism also is used to regulate expression of *yczA-ycbK*, the operon of *B. subtilis* mentioned above, that responds to tRNATrp, and regulates *trp* operon expression. We can only speculate why four different attenuation mechanisms are used to regulate tryptophan metabolism in different organisms.

DNA Microarray Analysis of *trp* Gene Expression in *E. coli*

Having devoted many years to studying virtually every aspect of tryptophan metabolism in *E. coli*, I have often wondered, what have we missed? Are there significant features of tryptophan metabolism that we have not recognized, and if so, are there unsuspected genes that play an important role? These questions can at last be partially addressed, by applying DNA microarray technology. We can now measure the mRNA (and soon protein) levels corresponding to every gene, under all physiological conditions we choose to examine. This technology, and its application to several organisms, including *E. coli*, has been highly developed in the labs of Patrick Brown and David Botstein of our Medical School, in collaboration with Nicholas Cozzarelli's group and others in the San Francisco

Bay area. Fortunately for me, a postdoc working with Brown and Botstein, Arkady Khodursky, was interested in applying their microarray technology to tryptophan metabolism in *E. coli*. Using this approach, we detected some 200 genes that exhibit increased or decreased expression, in response to tryptophan starvation, upon addition of excess tryptophan to the growth medium, or when we inactivated the *trp* repressor. It was very comforting to find that only genes of operons known to be regulated by tryptophan, such as the genes of the *trp* repressor regulon and the TyrR regulon, responded comparably. Similarly, no gene other than the two genes of the tryptophan-inducible *tna* degradative operon displayed the same expression pattern. As expected, many operons were transcriptionally activated or inhibited when tryptophan starvation was imposed or tryptophan was added. However, none of these genes exhibited an expression pattern suggesting their direct regulation by tryptophan. Equally pleasing was the finding that quantitative estimates of expression of the genes of the *trp* operon agreed within a factor of two with expectations based on prior enzyme activity measurements. On the basis of these analyses, we believe we have identified most of the major genes of *E. coli* that are directly involved in tryptophan metabolism under our growth conditions (105a).

Analysis of the Sluggish Low-Temperature Activity of a *trp* Enzyme from a Thermophile

A second project we have worked on recently also is based on the application of new technology. Kasper Kirschner, a longtime friend and collaborator, has devoted many years analyzing structure/function relationships and catalytic mechanisms for the tryptophan pathway enzymes. He and his colleague, JN Jansonius, and their coworkers have solved the structures of several tryptophan pathway enzymes, including InGP synthase from both *E. coli* and the thermophilic bacterium *Sulfolobus sulfataricus*. As is typical of enzymes from thermophiles, its InGP synthase is only slightly active at 37°C and lower temperatures. Comparison of the structures of the enzyme from these two sources did not reveal the features of the thermophilic enzyme responsible for its catalytic sluggishness. To explore the basis of this inactivity, M-C Yee of my group used Willem Stemmer's DNA shuffling procedure to introduce numerous amino acid changes, and combinations of such changes, in the *S. sulfataricus* TrpC protein, increasing the enzyme's activity at low temperatures. Kinetic/catalytic analyses were then performed with purified "active" mutant and parental enzymes by Astrid Metz and others in the Kirschner group. Their findings indicate that the catalytic sluggishness of the thermophilic enzyme at low temperatures is due to its unusually high affinity for the product of the reaction, InGP (106). Slow release of the product from the enzyme's active site apparently "constipates" the enzyme. The DNA shuffling procedure facilitated our ability to produce and combine individual mutational changes to yield proteins with increased catalytic activity at low temperatures.

FAMILY MATTERS

I feel extremely fortunate to have had supportive parents, brother, sister, teachers, mentors, and fellow scientists. My deceased wife, Carol, was particularly helpful and understanding. During the more than 40 years we were together, Carol recognized my dedication to science and made every effort to ensure that despite this commitment our lives together would be as thoroughly enjoyable as possible. I believe I participated actively in bringing up our three sons, but Carol assumed this as her primary responsibility. Breast cancer took her life in her early 60s. Fortunately, she did live to enjoy the marriage of our three sons, and the birth of three granddaughters. Our oldest and youngest sons, Steve and Marty, are practicing scientists, and our other son, Bob, sells scientific equipment. Marty's work on flowering and fruit formation in Arabidopsis is spectacular! A review describing his studies and those of others is in the 1999 *Annual Review of Biochemistry* (107). I do not recall ever going out of my way to persuade any of my sons to pursue a career in science. In 1992 I remarried to Edna Crawford, widow of my former postdoc, coworker, and close friend, Irving Crawford. Edna, too, understands, accepts, and enjoys my love of science. She is determined to keep me working and happy. I am doing my best to help her enjoy our life together.

OTHER ACTIVITIES

As a concerned member of the Department of Biological Sciences at Stanford for more than 40 years, I contributed to faculty recruitment and decision making in my department and in other units of our university. I participated in the rebuilding of our department, and relished sharing my thoughts on the future with Cliff Grobstein, David Perkins, Paul Ehrlich, Donald Kennedy, Norman Wessells, Robert Schimke, Allan Campbell, Phillip Hanawalt, and Robert Simoni. I have often discussed university affairs with Paul Berg, Dale Kaiser, David Hogness, Patricia Jones, Lucy Shapiro, and Channing Robertson. I taught in two major undergraduate courses at different periods in my career. One was our initial core molecular biology course, taught with Simoni, and the second was an intermediate molecular biology course, taught with Schimke, and more recently, with Joseph Lipsick. I offered two graduate-level courses, entitled Microbial Genetics and Gene Action. Many of the students who took one or both of these courses informed me years later that I had a positive impact on their careers. I also directed a departmental National Institutes of Health (NIH) Training Grant in Biochemical Genetics for more than 20 years. I was appointed Herzstein Professor of Biology at Stanford in the 1960s. Approximately 25 graduate students and 60 postdoctoral fellows have trained with me. Many of the postdoctoral fellows who were in my group received their support from worthy funding organizations. I have received generous research support from the NIH, National Science Foundation (NSF), American Cancer Society, and American Heart Association (AHA). In 1969 I was appointed

a Career Investigator of the AHA, a position I held for 26 years. I served as president of the Genetics Society of America and of the American Society of Biological Chemists. I also served on several NIH, NSF, AHA, and National Academy of Science panels, and on advisory boards for the research programs of fellow scientists, such as the program of my good friend, Stanley Prusiner. One event I have always enjoyed is the annual or biannual Tryptophan Plus Conference, generally held at Asilomar (Pacific Grove, California). Scientists who work on tryptophan metabolism or related topics generally attend this meeting and present their research findings. This meeting has always been great fun, and very exciting.

In the early 1980s Alex Zaffaroni, a scientist and prominent developer of biotechnology companies, invited me to join him, Arthur Kornberg, and Paul Berg in establishing a new biotech company, to be named the DNAX Research Institute. DNAX's initial mission was to discover new principles and materials in the basic sciences that could lead to useful commercial applications. After a few years of operation, DNAX was purchased by the Schering-Plough Corporation, and the research being conducted assumed broader objectives, mostly in the area of immunology. All aspects of this relationship have been thoroughly enjoyable, particularly getting to know the scientists at DNAX and interacting with Alex, Arthur, and Paul, and the leadership at Schering-Plough.

CAREER ACCOMPLISHMENTS: COPING WITH EGO, RECOGNITION, AND REALITY

Looking over the contributions from my lab, most modern-day scientists would probably agree that our research provided two major scientific advances—establishing gene-protein colinearity, and elucidating the features of operon regulation by transcription attenuation. Let me emphasize again that other investigators contributed significantly to the solution of each of these problems. In my opinion, this is how scientific advancement should proceed. We should never set our goals so high that we ignore making minor contributions. It is often the combination of these advances that points the way to the final solution to a problem. I believe that everyone participating in a discovery, regardless of the stage at which he or she contributed, should be pleased by its outcome. Unfortunately, as scientific knowledge increases, the temporal impact of important early discoveries is frequently forgotten because they become incorporated into a foundation of facts that serves as the basis for ongoing research. Thus, we now determine a protein sequence exclusively by predictions from a DNA sequence; the existence of gene-protein colinearity is taken for granted! Although it is difficult to accept, each of us should realize that science constantly moves ahead.

I became a scientist because I found science to be exceptionally exciting. I loved having the opportunity to make original contributions. Aiding young scientists to find their way proved to be an extraordinary bonus I did not anticipate. It has been a great pleasure summarizing my career activities in this article; I enjoyed reflecting

on the contributions of the many talented individuals who joined me in exploring the unknown.

ACKNOWLEDGMENTS

I would like to express my appreciation to all the individuals who have influenced the events in my life and have contributed to my scientific accomplishments. I would like to thank Howard Gest and Robert Simoni in particular for their excellent comments and helpful suggestions regarding this review.[1]

LITERATURE CITED[1]

1. Markert C. 1950. *Genetics* 35:60–75
2. Umbreit WW, Wood WA, Gunsalus IC. 1946. *J. Biol. Chem.* 165:731–32
3. Yanofsky C. 1952. *Proc. Natl. Acad. Sci. USA* 38:215–26
4. Yanofsky C. 1956. In *Enzymes: Units of Biological Structure and Function*, ed. OH Gaebler, pp. 147–60. New York: Academic
5. Suskind SR, Yanofsky C, Bonner DM. 1955. *Proc. Natl. Acad. Sci. USA* 41:577–82
6. Yanofsky C. 1954. *J. Bacteriol.* 68:577–84
7. Penfound T, Foster JW. 1996. In *E. coli and Salmonella: Cellular and Molecular Biology*, ed. FC Neidhardt, R Curtiss III, JL Ingraham, ECC Lin, KB Low, et al. 1:721–30. Washington, DC: Am. Soc. Microbiol.
8. Yanofsky C. 1955. *Biochim. Biophys. Acta* 16:594–95
9. Yanofsky C. 1956. *Biochim. Biophys. Acta* 20:438–39
10. Smith OH, Yanofsky C. 1960. *J. Biol. Chem.* 235:2051–57
11. Doy CH, Gibson F. 1959. *Biochem. J.* 72:586–97
12. Yanofsky C, Lennox ES. 1959. *Virology* 8:425–47
13. Yanofsky C. 1959. *Biochim. Biophys. Acta* 31:408–16
14. Yanofsky C, Rachmeler M. 1958. *Biochim. Biophys. Acta* 28:640–41
15. Srere PA. 2000. *Trends Biochem. Sci.* 25:150–53
16. Hyde CC, Ahmed SA, Padlan EA, Miles EW, Davies DR. 1988. *J. Biol. Chem.* 263:17857–71
17. Crawford IP, Yanofsky C. 1958. *Proc. Natl. Acad. Sci. USA* 44:1161–70
18. Creighton TE, Yanofsky C. 1966. *J. Biol. Chem.* 241:980–90
19. Goldberg M, Creighton TE, Baldwin R, Yanofsky C. 1966. *J. Mol. Biol.* 21:71–82
20. Crawford IP, Yanofsky C. 1959. *Proc. Natl. Acad. Sci. USA* 45:1280–87
21. Helinski D, Yanofsky C. 1962. *Proc. Natl. Acad. Sci. USA* 48:173–83
22. Henning U, Yanofsky C. 1962. *Proc. Natl. Acad. Sci. USA* 48:1497–504
23. Guest JR, Drapeau GR, Carlton BC, Yanofsky C. 1967. *J. Biol. Chem.* 242:5442–46

[1]I have written two previous reviews summarizing our accomplishments, one focused on gene-protein colinearity, and the second reviewing transcription attenuation: Yanofsky C. 1976. The search for the structural relationship between gene and enzyme. In *Reflections on Biochemistry*, ed. A Kornbert, BL Horecker, L Cornudella, J Oro, pp. 263–71. New York: Pergamon Press; Yanofsky C. 2000. Transcription attenuation: once viewed as a novel regulatory strategy. *J. Bacteriol.* 182:1–8.

24. Yanofsky C, Carlton BC, Guest JR, Helinski DR, Henning U. 1964. *Proc. Natl. Acad. Sci. USA* 51:266–72
25. Yanofsky C, Drapeau GR, Guest JR, Carlton BC. 1967. *Proc. Natl. Acad. Sci. USA* 57:296–98
26. Sarabhai AS, Stretton ADW, Brenner S, Bolle A. 1964. *Nature* 201:13–16
27. Berger H, Brammar WJ, Yanofsky C. 1968. *J. Mol. Biol.* 34:219–38
28. Guest JR, Yanofsky C. 1966. *Nature* 210:799–802
29. Cox EC, Yanofsky C. 1967. *Proc. Natl. Acad. Sci. USA* 58:1895–902
30. Crow JE. 2000. *Genetics* 154:955–58
31. Helinski DR, Yanofsky C. 1963. *J. Biol. Chem.* 238:1043–48
32. Brody S, Yanofsky C. 1963. *Proc. Natl. Acad. Sci. USA* 50:9–16
33. Carbon J, Berg P, Yanofsky C. 1966. *Proc. Natl. Acad. Sci. USA* 56:764–71
34. Gupta N, Khorana HG. 1966 *Proc. Natl. Acad. Sci. USA* 56:772–79
35. Murgola EJ, Yanofsky C. 1974. *J. Mol. Biol.* 86:775–84
36. Franklin NC, Dove WF, Yanofsky C. 1965. *Biochem. Biophys. Res. Commun.* 18:898–909
37. Imamoto F, Ito J, Yanofsky C. 1966. *Cold Spring Harbor Symp. Quant. Biol.* 31:235–49
38. Morse DE, Yanofsky C. 1970. *RNA-Polymerase and Transcription, Proc. 1st Int. Lepetit Colloq., Florence, 1969*, 1:204–7. Amsterdam: North-Holland
39. Morse DE, Mosteller RD, Yanofsky C. 1969. *Cold Spring Harbor Symp. Quant. Biol.* 34:725–39
40. Rose JK, Mosteller RD, Yanofsky C. 1970. *J. Mol. Biol.* 51:541–50
41. French S, Martin K, Patterson T, Bauerle R, Miller OL Jr. 1985. *Proc. Natl. Acad. Sci. USA* 82:4638–42
42. Baker RF, Yanofsky C. 1968. *Nature* 218:26–29
43. Morse DE, Mosteller RD, Baker RF, Yanofsky C. 1969. *Nature* 223:40–43
44. Hardman JK, Yanofsky C. 1965. *J. Biol. Chem.* 240:725–32
45. Jackson DA, Yanofsky C. 1969. *J. Biol. Chem.* 244:4523–38
46. Jackson DA, Yanofsky C. 1969. *J. Biol. Chem.* 244:4539–46
47. Spudich JA, Horn V, Yanofsky C. 1970. *J. Mol. Biol.* 53:49–67
48. Herschfield V, Boyer HW, Yanofsky C, Lovett MA, Helinski DR. 1974. *Proc. Natl. Acad. Sci. USA* 71:3455–59
49. Rose JK, Squires CL, Yanofsky C, Yang HL, Zubay G. 1973. *Nature New Biol.* 245:133–37
50. Doolittle WF, Yanofsky C. 1968. *J. Bacteriol.* 95:1283–94
51. Baker R, Yanofsky C. 1972. *J. Mol. Biol.* 69:89–102
52. Jackson EN, Yanofsky C. 1973. *J. Mol. Biol.* 76:89–101
53. Kasai T. 1974. *Nature.* 279:523–27
54. Imamoto F. 1968. *Nature.* 220:31–34
55. Hiraga S, Yanofsky C. 1973. *J. Mol. Biol.* 79:339–49
56. Bertrand K, Yanofsky C. 1976. *J. Mol. Biol.* 103:339–49
57. Bertrand K, Korn LJ, Lee F, Yanofsky C. 1977. *J. Mol. Biol.* 117:227–47
58. Squires C, Lee F, Bertrand K, Squires CL, Bronson MJ, Yanofsky C. 1976. *J. Mol. Biol.* 103:351–80
59. Lee F, Yanofsky C. 1977. *Proc. Natl. Acad. Sci. USA* 74:4365–69
60. Platt T, Squires C, Yanofsky C. 1976. *J. Mol. Biol.* 103:411–20
61. Zurawski G, Elseviers D, Stauffer GV, Yanofsky C. 1978. *Proc. Natl. Acad. Sci. USA* 75:5988–92
62. Oxender DL, Zurawski G, Yanofsky C. 1979. *Proc. Natl. Acad. Sci. USA* 76:5524–28
63. Yanofsky C, Soll L. 1977. *J. Mol. Biol.* 113:663–77
64. Ito J, Cox EC, Yanofsky C. 1969. *J. Bacteriol.* 97:725–33
65. Ito J, Yanofsky C. 1969. *J. Bacteriol.* 97:34–42

66. Bennett GN, Schweingruber ME, Brown KD, Squires C, Yanofsky C. 1976. *Proc. Natl. Acad. Sci. USA* 73:2351–55
67. Oppenheim DS, Yanofsky C. 1980. *J. Mol. Biol.* 144:143–61
68. Gunsalus RP, Zurawski G, Yanofsky C. 1979. *J. Bacteriol.* 140:106–13
69. Gunsalus RP, Yanofsky C. 1980. *Proc. Natl. Acad. Sci. USA* 77:7117–21
70. Singleton CK, Roeder WD, Bogosian G, Somerville RL, Weith HL. 1980. *Nucleic Acids Res.* 8:1551–60
71. Kelley RL, Yanofsky C. 1985. *Proc. Natl. Acad. Sci. USA* 82:483–87
72. Graddis TJ, Klig LS, Yanofsky C, Oxender DL. 1988. *Proteins: Struct. Funct. Genet.* 4:173–81
73. Klig LS, Carey J, Yanofsky C. 1988. *J. Mol. Biol.* 202:769–77
74. Kumamoto AA, Miller WG, Gunsalus RP. 1987. *Genes Dev.* 1:556–64
75. Lawson CL, Carey J. 1993. *Nature* 366:178–82
76. Winkler ME, Yanofsky C. 1981. *Biochemistry* 20:3738–44
77. Fisher RF, Das A, Kolter R, Winkler ME, Yanofsky C. 1998. *J. Mol. Biol.* 182:397–409
78. Winkler ME, Mullis K, Barnett J, Stroynowski I, Yanofsky C. 1982. *Proc. Natl. Acad. Sci. USA* 79:2181–85
79. Stroynowski I, Yanofsky C. 1982. *Nature* 298:34–8
80. Fisher RF, Yanofsky C. 1983. *J. Biol. Chem.* 258:8146–50
81. Landick R, Carey J, Yanofsky C. 1985. *Proc. Natl. Acad. Sci. USA* 82:4663–67
82. Roesser JR, Nakamura Y, Yanofsky C. 1989. *J. Biol. Chem.* 264:12284–88
83. Hall C, Yanofsky C. 1982. *J. Bacteriol.* 151:918–23
84. Schneider WP, Nichols BP, Yanofsky C. 1981. *Proc. Natl. Acad. Sci. USA* 78:2169–73
85. Yanofsky C, Platt T, Crawford IP, Nichols BP, Christie GE, et al. 1981. *Nucleic Acids Res.* 9:6647–68
86. Oppenheim DS, Yanofsky C. 1980. *Genetics* 95:785–95
87. Das A, Yanofsky C. 1984. *Nucleic Acids Res.* 12:4757–68
88. Selker EU, Yanofsky C, Driftmeier K, Metzenberg RL, Aizner-DeWeerd B, Raj-Bhandary UL. 1981. *Cell* 24:819–28
89. Vollmer SJ, Yanofsky C. 1986. *Proc. Natl. Acad. Sci. USA* 83:4869–73
90. Berlin V, Yanofsky C. 1985. *Mol. Cell. Biol.* 5:849–55
91. Orbach MJ, Vollrath D, Davis RW, Yanofsky C. 1988. *Mol. Cell. Biol.* 8:1469–73
92. Glass NL, Vollmer SJ, Staben C, Grotelueschen J, Metzenberg RL, Yanofsky C. 1988. *Science* 241:570–73
93. Kuroda MI, Shimotsu H, Henner DJ, Yanofsky C. 1986. *J. Bacteriol.* 167:792–98
94. Henner D, Yanofsky C. 1993. In Bacillus subtilis *and Other Gram-Positive Bacteria: Biochemistry, Physiology, and Molecular Genetics*, ed. AL Sonenshein, JA Hoch, R Losick, pp. 269–80. Washington, DC: Am. Soc. Microbiol.
95. Gollnick P, Ishino S, Kuroda MI, Henner DJ, Yanofsky C. 1990. *Proc. Natl. Acad. Sci. USA* 87:8726–30
96. Babitzke P, Bear DG, Yanofsky C. 1995. *Proc. Natl. Acad. Sci. USA* 92:7916–20
97. Antson AA, Otridge J, Brzozowski AM, Dodson EJ, Dodson GG, et al. 1995. *Nature* 374:693–700
98. Antson AA, Dodson EJ, Dodson G, Greaves RB, Chen X, Gollnick P. 1999. *Nature* 401:235–42
99. Merino EP, Babitzke P, Yanofsky C. 1995. *J. Bacteriol.* 177:6362–70
100. Lee AI, Sarsero JP, Yanofsky C. 1996. *J. Bacteriol.* 178:6518–24
101. Sarsero J, Merino E, Yanofsky C. 2000. *Proc. Natl. Acad. Sci. USA* 97:2656–61
102. Deeley MC, Yanofsky C. 1981. *J. Bacteriol.* 147:787–96

103. Stewart VJ, Yanofsky C. 1985. *J. Bacteriol.* 164:713–40
104. Konan KV, Yanofsky C. 2000. *J. Bacteriol.* 182:3981–88
105. Grundy FJ, Henkin T. 1994. *J. Mol. Biol.* 235:798–804
105a. Khodursky AB, Peter BJ, Cozzarelli NR, Botstein D, Brown PO, Yanofsky C. 2000. *Proc. Natl. Acad. Sci. USA.* 97: 12170–75
106. Merz A, Yee MC, Szadkowski H, Pappenberger G, Crameri A, et al. 2000. *Biochemistry* 39:880–89
107. Ferrándiz C, Pelaz S, Yanofsky MF. 1999. *Annu. Rev. Biochem.* 68:321–54