Pastel portrait by Patricia Lomasney Roberts

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A Tale of Good Fortune in the Era of DNA

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Abstract

Two strains of good fortune in my career were to stumble upon the Watson–Gilbert laboratory at Harvard when I entered graduate school in 1964, and to study gene regulation in bacteriophage λ when I was there. λ was almost entirely a genetic item a few years before, awaiting biochemical incarnation. Throughout my career I was a relentless consumer of the work of previous and current generations of λ geneticists. Empowered by this background, my laboratory made contributions in two areas. The first was regulation of early gene transcription in λ, the study of which began with the discovery of the Rho transcription termination factor, and the regulatory mechanism of transcription antitermination by the λ N protein, subjects of my thesis work. This was developed into a decades-long program during my career at Cornell, studying the mechanism of transcription termination and antitermination. The second area was the classic problem of prophage induction in response to cellular DNA damage, the study of which illuminated basic cellular processes to survive DNA damage.

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BEGINNINGS

A war baby, of a war marriage, I traveled often in early years with my mother from home in Michigan to ports of call of my father's navy ship, a destroyer escort guarding convoys in the Atlantic. I think my parents barely knew each other then, but they started a successful marriage after the war in Chicago, where my father sought a civilian profession as a photographer. No opportunity arising, we migrated when I was about three to my father's home in Texas, where he applied to graduate school in English at the University of Texas, financed by the G.I. Bill. He considered geology but found that a PhD in English would be quicker. He stayed for his career at Texas and became a Professor of English, specializing in English and American novels, and particularly the English novelist D.H. Lawrence (affectionately referred to by my mother as Dirty Herbert; she also named her dog Lawrence). As a protégé of the eventual Chancellor Harry Ransom, he was intensely involved in establishing collections of important literary materials, a venture enabled by oil money from generous donors. He was for years Director of the Humanities Research Center, which later became the Harry Ransom Center. Thus, I grew up in Austin as a faculty brat, interrupted by a two-year interlude in Norfolk, Virginia, when my father was called up for the Korean War, and two years in Marina di Pisa, Italy, where we lived during his Fulbright fellowship to teach literature at the University of Pisa.

The stay in Italy was a brave and transformative adventure. We had no money except the monthly Fulbright check, and of course nobody spoke Italian in the beginning. But my parents used all of their resources for constant travel and exploration of Europe and Britain; one trip in our used Fiat Millecento nearly ended when there was just barely enough money remaining to repair a broken water pump in Genoa. I learned Italian considerably better than they, despite attending a nearby US army school that functioned only in English, and I occasionally translated for my father in his searches for literary materials; once he found a treasure trove of manuscripts and annotated books left by D.H. Lawrence during a stay in Florence.

UNIVERSITY OF TEXAS, 1960–1964

Deemed too young to travel far after graduating from high school at age 16, I enrolled in the University of Texas and in fact lived at home for the four years. Therefore, I was fledged only when I went to graduate school, years after everyone else, somewhat of a social misfit but eager to engage the world.

The education I had at Texas was very good. The program I followed combined science with a selective liberal arts program named Plan II (the facetious motto for which was "I plan to, but never get around to it"). I was taught by distinguished scholars of the time who had been attracted to Texas—John Silber, William Arrowsmith, Roger Shattuck. Initially my science was to be physics, because the traditional high school order was biology, chemistry, physics, so physics must be the most challenging. I did in the end get a rather thin degree in physics, but early on I had decided that biology was my real interest. This was due to one of my major influences at this time, Professor Irwin Spear, who taught the enriched freshman biology class of the Plan II program. It was an excellent course in which I did well, and Spear hired me as a counselor for high school summer biology programs. He suggested that biophysics might be the right subject, and that name stuck. I went to the library to try to figure out what biophysics might be, and I remember finding a book by a Russian about analyzing automobile traffic patterns in Chicago. Later I found more amenable aspects of biophysics.

The second important influence in Texas was Wilson Stone and the Genetics Foundation at the University of Texas, site of my first research experiences. Stone was a Texan, born in the same remote western town of Junction where my father grew up. Rejecting his parents' desire for him to be a Baptist preacher, Stone enrolled in the University of Texas, which already had distinguished biologists. He was a student initially of H.J. Müller, who discovered mutagenesis by radiation, and whose purported X-ray machine was displayed in the Experimental Science Building where I worked at Texas. Müller was a student of T.H. Morgan, so I have a (very indirect) connection to the origins of modern genetics. Stone's work was in *Drosophila* genetics, and particularly the study (with J.T. Patterson) of translocations, and eventually of the evolution of *Drosophila* species. But he had an interest in radiation biology, having discovered that radiation of bacterial medium without the bugs made it mutagenic—a subject in which I assisted as an undergraduate aide, thus becoming familiar with bacteria and phage. T.S. Painter discovered (or rediscovered) *Drosophila* giant salivary chromosomes at Texas at this time, a critical resource in cytogenetics. The early geneticist A.H. Sturtevant, a student of T.H. Morgan's and founder of the Division of Biology at Caltech, gave a series of lectures in about 1964 on the history and professional pedigrees of genetics—to which I unfortunately paid only scant attention.

HARVARD 1964–1970

Graduating with a good record into a favorable market for graduate students, I picked Harvard over Caltech and Stanford. Caltech actually frightened me away by promising to put me into a

research laboratory immediately, for which I knew I had little preparation, whereas the Harvard biophysics program offered a full set of courses over nearly two years. I was assigned to Walter Gilbert as an introductory advisor, a great stroke of luck because I became immediately interested in his research, which led to my joining the Watson–Gilbert laboratory.

The Watson–Gilbert Laboratory

The structure and culture of this laboratory were unique. Founded by James D. Watson only a few years after the structure of DNA was published, it focused on central processes of gene expression, particularly the mechanism of protein and RNA synthesis. Jim was joined by Wally Gilbert a few years later, continuing a friendship from Cambridge in the fifties. Wally was a Harvard theoretical physicist at this time, still teaching physics and advising physics graduate students while he worked in the laboratory, occasionally erasing gene names and images of genetic crosses from the tea-room blackboard in order for a physics student to fill it with equations. I entered the laboratory the summer after my first year, doing what is now called a rotation, and joined it permanently in my second year. At this time Wally, along with Benno Müller-Hill, was trying to purify the lactose repressor. After many attempts, and in parallel with Mark Ptashne's isolation of the bacteriophage λ repressor, they did detect, purify, and characterize the lactose repressor during the first several years of my graduate career. This example of success through relentless work after repeated failure was a foundational example to me about doing science.

Jim continued the English tradition of having students publish under their names alone. In fact, this did reflect a certain freedom that students had to make independent ventures, which I also did occasionally. But there was nonetheless strong direction and guidance in the laboratory, and relentless critical advice. Wally made essential contributions of experimental advice and insight to students who were officially Jim's, a contribution perhaps not appreciated from the outside at this time. I was Wally's first official student, I believe. The other critical member of the laboratory was Klaus Weber, who came as a postdoctoral fellow and became an Assistant Professor; his expertise in protein chemistry particularly influenced those of us purifying transcription factors.

It's worth remembering how close to the beginning of time this was. When I started in 1964, the genetic code was just being worked out; in Matt Meselson's genetics course we received a reprint of the famous Crick et al. paper (9), only a few years old, showing by proflavine mutagenesis that the code is a triplet. The Jacob and Monod paper (25) postulating regulation by repressors and operators was only three years old, and the existence of mRNA had just been shown. Enzymology of RNA and DNA synthesis was only emerging, led by Kornberg's isolation of *Escherichia coli* DNA polymerase I.

A central goal of the laboratory was understanding the mechanism of protein biosynthesis, using the genome of the *E. coli* RNA phage R17 as a messenger. We were in competition, a little contentious at times but not unfriendly, with Norton Zinder of Rockefeller University, the discoverer of RNA bacteriophage, whose laboratory employed the sister RNA phage f2. In the Watson–Gilbert laboratory Mario Capecchi identified the products of R17 translation in vitro, leading to the discovery (with Gary Gussin) that tRNA is the nonsense suppressor (7), and to the isolation of release factors acting at the termination step. Joan (Argetsinger) Steitz had just purified an essential single-copy structural protein of R17, the A protein, and she and I used this to increase the infectivity of phage reconstituted in vitro—my first sort of independent foray into experimentation (54). For my major project I made some abortive attempts to characterize mRNA instability, an essential element of the Jacob and Monod model of regulation, using labeled R17 RNA, but got nowhere.

Promoter Function In Vitro

At the time my fellow graduate student Richard Burgess was purifying RNA polymerase (RNAP) from *E. coli*, and I asked Wally if I might work on transcription as well. The notion of the promoter as an initiation site for RNAP was well established, but all evidence for promoter function was genetic. Wally knew of a mutation in λ (*c17*) that behaved like a new promoter, unregulated by λ repressor, and thought this might be detectable in vitro. Using Dick's enzyme I found this activity, the first biochemical evidence of promoter function; I detected increased transcription from the correct general region of the λ genome, with the rough measures we had then—hybrid phages and separated strands (47). An incidental consequence of this collaboration was my observation one day that Dick's new preparation of RNAP was mostly inactive in transcribing phage λ DNA. I didn't realize this meant anything except an inactive enzyme, but Dick with Andrew Travers established that an essential component of the active RNAP had been removed in a novel purification step on a phosphocellulose column: This was of course the discovery of the sigma factor (specifically σ^{70}), codiscovered by Ekkehard Bautz and John Dunn at Rutgers and published in a single paper with them (6). (Sigma was also codiscovered by Joseph Krakow of Hunter College using *Azotobacter*, but this was not published.)

Rho Termination Factor

The *c17* promoter mutation was critical to my main endeavor as a graduate student: the isolation and characterization of the bacterial termination factor Rho, which I discovered through a search in uninfected cell extracts for influences that increased the apparent fidelity of transcription. Wally was particularly interested in showing that RNAP could work accurately. One motivation was that attempts to show repression by lactose repressor had failed, although *lac* RNA identifiable by hybridization was made in vitro by RNAP preparations from DNA of a *lac*-transducing phage, possibly attributable to spurious random transcription. Of course, the main reason for this failure was the absence of the CAP (or CRP) activator, not yet discovered. Nonetheless, it is true that transcription of λ DNA by purified RNAP yields RNA for segments of the λ genome, such as the left half and the *b2* region, that should be silent in the absence of positive phage-encoded regulators like the *N* gene product, even without repressor. Therefore, I searched for factors that would enhance the modest effect of the *c17* mutation, presumably by reducing the spurious background. Addition of cell extract did in fact reduce background transcription (eventually; the first three attempts gave no effect or an effect in the wrong direction), thereby concentrating the *c17* promoter–specific RNA. The first variant experiment was to test dialyzed extract versus dialysate—was there a magic small-molecule factor? No, but the activity responsible for the effect turned out to be purifiable as a single protein that eventually was named Rho (48).

I had purified Rho to homogeneity before discovering its actual activity. I even tested whether it would act as an initiation factor like sigma, which had been identified about this time. The realization that Rho is a termination factor resulted from attempts to determine whether it suppressed aberrant initiation at nonpromoter sites. It was known that most mRNA in *E. coli* initiates with either ATP or GTP, and it seemed possible that this ratio might be shifted by an accuracy factor. Two double-label experiments were done to measure the effect of the factor on RNA initiation from λ DNA, using $\gamma-^{32}$P-labeled ATP or GTP, along with ^{3}H-labeled nucleoside triphosphates (NTPs) to monitor overall synthesis. Unexpectedly, the factor had no effect on initiation with either ATP or GTP, and in fact increased initiation a bit, but instead it depressed total RNA synthesis about threefold. This was consistent with sucrose gradient analysis that showed that the factor decreased the size of RNA products several-fold. The use of genetically defined rightward and leftward promoter mutants (λ*x* and λ*sex*) identified the slower and faster sedimenting peaks of

Rho-mediated RNA products as the products of rightward and leftward transcription. The simplest interpretation was that the factor (soon named Rho, for release) terminated RNA synthesis. Any generalized nuclease activity could be discounted by showing that presynthesized RNA was not degraded in reactions in which Rho was active to decrease the size of new transcripts, although this did not exclude a transcription-coupled exonuclease activity. Of course, I conjectured that Rho was a (the?) cellular transcription termination factor. This was correct, although a major mode of termination in the cell turned out to be the intrinsic terminator, a nucleotide sequence that requires no external factor. And the notion that an important function of Rho might be to prevent spurious transcription is more correct than was imagined at the time. Its major role is to suppress synthesis of RNA that is not being utilized: untranslated RNA, such as that synthesized after a nonsense codon releases ribosomes, or adventitious (antisense) transcription (40).

Proposal That λ *N* Gene Product Is an Antiterminator

The question then was, How would a termination activity preferentially decrease background synthesis? It was known that mRNA synthesis in the λ life cycle starts from the immunity region and goes in both directions, and that expression of genes outside the immunity region and gene *N* required (or mostly required) the activity of the *N* gene product itself. However, N is not present in the in vitro reaction, so that accurate transcription should not exist beyond these boundaries of the immunity region and the *N* gene (**Figure 1**). Thus, the Rho termination factor might establish the accurate condition by terminating synthesis at these boundaries, and this was shown to be where Rho acts, as nearly as possible with the methods available then (48). Furthermore, the likely activity of N protein then was to overcome these termination sites. Consistently, it was known from various published and unpublished experiments that N-stimulated synthesis beyond these boundaries required the function of the repressor-sensitive early promoters, arguing that N does not simply create a new promoter near the boundaries. From the genetics alone, it could have been argued that transcription from the immunity region might somehow open DNA downstream and make it susceptible to an activity of N that created a new promoter; a similar model was proposed for *Q* gene function because Q acted throughout the span of the entire phage late gene region, which seemed to be implausible (23). Or N might stabilize an unstable transcript, e.g., by stimulating coupled translation and simultaneously inhibiting mRNA degradation. However, the most attractive model was antitermination, originally called conditional termination: When transcription from promoters in the immunity region is influenced by N, the Rho-dependent termination sites in or near the immunity region are inactive (**Figure 1**). In fact, the size of the leftward in vitro synthesized RNA with Rho was appropriately about the same as that identified in vivo for *N*, and the rightward RNA could correspond to *cro* (*tof*), if its molecular weight were no more than about 10 kDa, as turned out to be the case. It also turned out that the prediction that Cro would not be controlled by N was true. An important later discovery by David Friedman was that the site of recognition preceded the site of action of N; thus, N modification of transcription, which occurs at a site named *nut*, for N utilization, is distinct from the antitermination event (13).

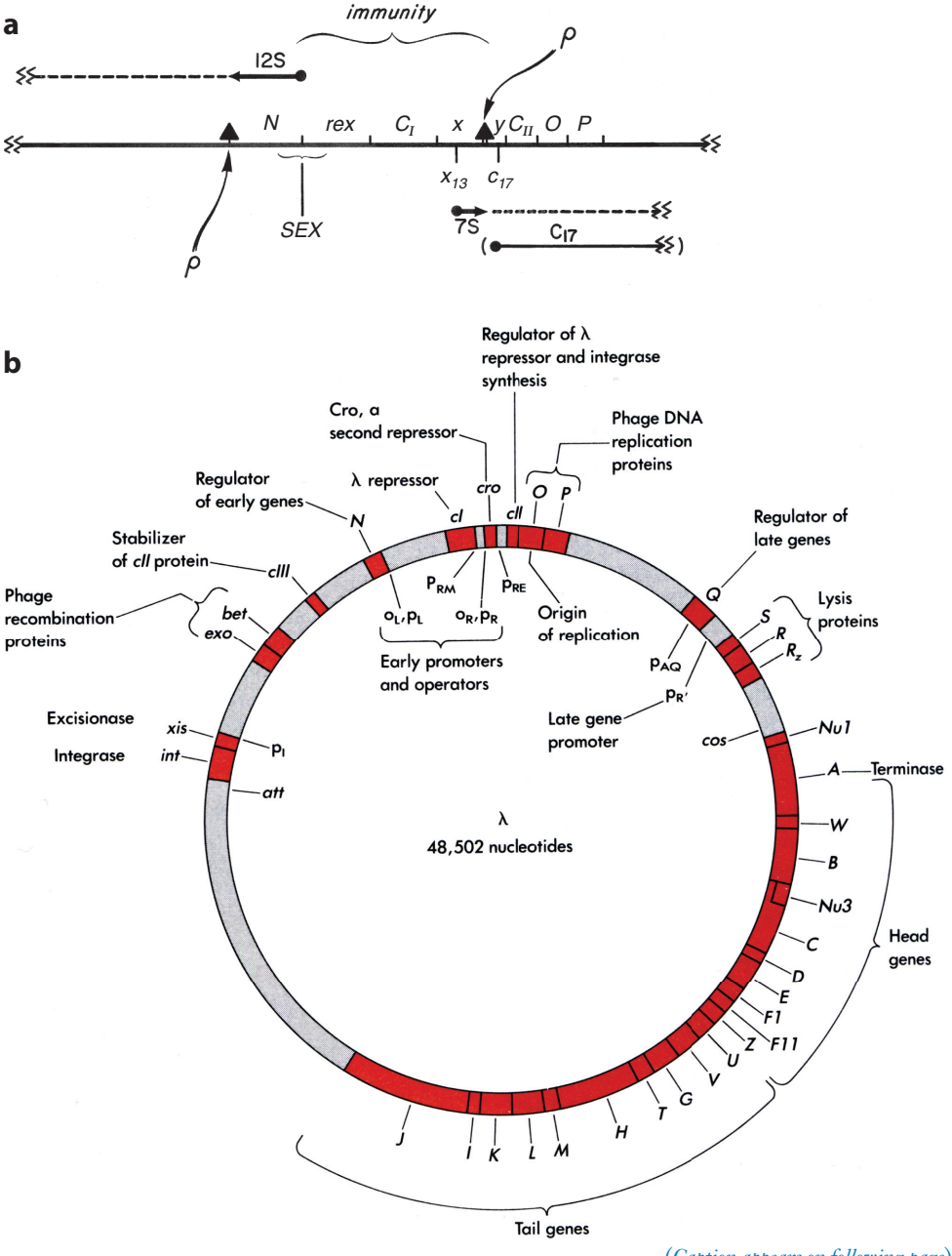
Oddly, my characterization of Rho might never have happened except for the Vietnam War, and the possibility of being drafted. An understanding draft board in Austin had accepted my request for an educational deferment, but if I had finished graduate school before my 26th birthday, in February 1970, I would have been in danger of conscription. Thus, I delayed finishing for six months or so, and it was during this time that the crucial experiments to characterize Rho function were done.

A year before I had married Christine Weiss, who had come to Boston from Wisconsin as a medical technologist and then became Wally Gilbert's technician at the time of the repressor

isolation. She and I became scientific partners, and she was our laboratory's majordomo for most of my career in Cornell; her endeavors are described further below.

CAMBRIDGE, ENGLAND, 1970–1971

A postdoctoral stay at the Medical Research Council (MRC) Laboratory in Cambridge in 1970–1971 was a formative exposure to a second germinal intellectual center of molecular genetics,



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Maps of bacteriophage λ. (*a*) The original map of λ in vitro transcription and model of Rho and λ N function proposed in Reference 48. Transcription in the presence of Rho produced transcripts sedimenting at about 7S and 12S, corresponding to RNAs of about 100 and 250 kDa. The mapping depended importantly on the effect of two promoter mutations, λ*x13* and λ*sex*, known to prevent rightward and leftward transcription, respectively, from the immunity region, and shown in Reference 48 to prevent synthesis of the 7S and 12S RNAs. These promoters are now named p_R and p_L. The site of the *C17* mutation is shown. *rex* is a gene expressed in the lysogen along with repressor, the product of gene *cI*. Panel adapted with permission from Reference 48. (*b*) A later and still current λ map from *Molecular Biology of the Gene* (63) showing sites and functions of critical λ genes, including the antiterminator Q. Gene *Q* is transcribed from p_R, and its product affects a transcript from the adjacent late gene promoter $p_{R'}$. Antitermination of $p_{R'}$ transcription at a terminator 200 bp downstream of $p_{R'}$ opens a gate to transcription of the entire 26-kB late gene operon comprising mostly structural protein genes of the capsid. Panel adapted with permission from Reference 63.

probably the center of the world in fact. Francis Crick and Sydney Brenner were consistently brilliant mentors and discussants, at a time when bacterial molecular genetics was being left behind for forays into complex organisms, most importantly of course the nematode, around which Brenner created an entirely new branch of science. Fred Sanger had developed RNA sequencing at the time, and I first planned to sequence the λ RNAs I had made in vitro for my thesis work. However, this proved too cumbersome when it was necessary to undertake a complex synthesis of all four radioactive NTPs, and I abandoned sequencing to start a relentlessly unsuccessful attempt to detect and purify the λ N protein with an extract-based transcription assay. This year and a half in England was filled with pleasant activities and diversions—talking science and buying antiques with Mark Bretscher, learning to fly gliders with the Cambridge University Gliding Club, and extensive touring in Europe. Uncomfortably, Chris was unable to work, and we needed to get back to a more structured life.

HARVARD 1971–1974

The λ Genes *N* and *Q* Antiterminators

I continued studies begun at the MRC when I returned to Harvard as a Junior Fellow in 1971, also returning to the Watson–Gilbert laboratory. The initial time was fruitless, as the N activity continued to resist my approaches. It was eventually detected in the laboratory by graduate student Jack Greenblatt, using the coupled in vitro system of Geoffrey Zubay, in which template DNA is transcribed and the resulting mRNA translated in a coupled reaction; this also was instrumental in identifying the activity of CAP protein and the arabinose C protein transcription activator (19). Later in this period I showed that a short in vitro transcript of the λ late region arose from the genetically defined site where transcription is stimulated by the λ Q protein (23), the subject of a major laboratory direction when I moved to Cornell. This small terminated transcript could reflect another antitermination mechanism, as suggested by Jim Dahlberg and Fred Blattner at the University of Wisconsin; they also succeeded in sequencing the λ early RNAs that I had found by in vitro synthesis (4).

λ Repressor Cleavage and the Cellular Response to DNA Damage

At this time Chris Roberts started working with me, starting a partnership that continued until she died of a rare neurological disease (multiple system atrophy) in 2009. Our important joint venture at Harvard was to look for the mechanism of induction of bacteriophage λ, understanding of which promised insights into important cellular processes. The famous experiments of Andre Lwoff et al. (35) had shown that ultraviolet irradiation (as well as other DNA-damaging

treatments) induces prophage growth en masse in a culture of lysogenic *Bacillus megaterium*, a process that also occurs spontaneously at a small but significant rate during normal growth; this discovery related λ induction to the cellular response to DNA damage, eventually named the SOS response (42, 64). Both Hideo Shinagawa in Japan and Paul Chadwick in Mark Ptashne's lab had shown that the specific operator binding activity of λ repressor in extracts disappears after the inducing treatment—arguing against the default model of a small-molecule inducer, as for the lactose operon. How otherwise might a protein be inactivated? One way would be covalent modification, and a specific precedent was the phage T4–induced adenylation of RNAP subunits; curiously, this was well before the pervasive role of covalent protein modification by phosphorylation in eukaryotic cellular regulation was known. Chris Goff and Bob Horvitz had studied this T4 process in the Watson–Gilbert lab (14, 24), and Chris Roberts and I made an attempt, initially along with Bob, to determine whether a ^{32}P label is incorporated into the λ repressor polypeptide after an inducing treatment. We intended to identify repressor on gels by antirepressor antibody precipitation of ^{35}S-labeled total protein, but the initial experiments showed that the polypeptide disappeared from the cell upon induction, giving rise to a fragment about half its size; presumably a second fragment was degraded further (51). Was proteolysis, then, the mechanism of induction? Proteolysis clearly could be the consequence of some other modification, and likely only biochemistry would give a satisfactory answer. The main genetic background of the problem was the requirement of the bacterial RecA protein for lysogenic induction. Although it was attractive to imagine some deep connection between prophage induction by DNA damage and the involvement of a cellular protein required for recombination and recombinational DNA repair, no plausible mechanism was apparent. However, this did not inhibit us from speculating that RecA might possibly be a protease itself (51).

JOB SEARCHING

By the time I was looking for a faculty position around 1973, my version of basic molecular biology of bacterial systems was not the greatest fancy; clearly, the job market was anticipating the emerging assault on eukaryotic systems. Several interviews failed, and one has stuck in memory. The University of California, Berkeley, Department of Chemistry wanted some sort of tame biologist in order to connect with the new biological world, although I suspect that the desire was by no means unanimous in the department. I interviewed for this position, meeting a series of rigidly traditional chemists. I recall that one asked if I could teach freshman chemistry; "I probably could," I replied, "but I can't imagine why you would want me to." No job offer, of course. I did receive an attractive offer from Cornell, facilitated no doubt by my acquaintance with Gerry Fink after taking the famous Cold Spring Harbor yeast genetics course. (I failed to be converted to yeast biology, but my understanding of genetics was immensely enriched by the course.) Gerry, Ray Wu, Joe Calvo, and Elizabeth Keller were welcoming, simpatico biologists when I arrived at Cornell in the fall of 1974.

CORNELL 1974–2020

My appointment was in the Section of Biochemistry, Molecular and Cell Biology at Cornell, where I have spent the last 45 years. This section (which elsewhere would be called a department) was centered when I arrived on Efraim Racker, an imperious mover-shaker from the era when giants of biochemistry roamed the Earth. As a specialist in oxidative phosphorylation, he studied components of cells that arrogant molecular biologists centrifuged away and discarded in order to get to the interesting soluble stuff (which I probably agreed with, although I've reformed significantly since then). Racker had been brought in as a New York State–supported Albert Einstein Professor, accompanied by abundant resources and positions to fill. The section contained several of Racker's

former postdocs, along with some senior scientists of distinction (Leon Heppel, Quentin Gibson, George Hess). But I was probably the first actually chosen in some section-wide democratic process and was certainly in an area out of section traditions. One advantage was that students were attracted to the exciting emerging science of molecular biology, and I quickly had a laboratory of five graduate students together with Chris and me, all sardined into a single small room in Wing Hall. Racker's attempt to keep a large adjacent laboratory empty at this time, in the hopes that his protégé Gottfried Schatz would return from Europe, was foiled by a visiting Chair and joint section member from the School of Nutrition who gave the space to me; we spread out in luxury in the laboratory I occupied until the Biotechnology Building was completed in 1989.

At Cornell we initiated studies in two areas based on the biology of bacteriophage λ: the mechanism of prophage induction in response to DNA damage, and the mechanism of transcription antitermination by the *Q* gene product in λ late gene transcription. These were subjects of my initial National Institutes of Health grant, and of its continuation as my only research grant for the next 40 years.

Repressor Cleavage, RecA Protein, and the Mechanism of SOS Gene Induction

Our initial and further work on induction uncovered a basic step in regulation of DNA repair functions, showing how the *recA* gene product is the critical element in the cellular response to DNA damage and λ prophage induction (52). In particular, RecA inactivates and directs cleavage of not only λ repressor (52) but also (as later shown) the cellular LexA repressor of genes involved in response to DNA damage, the SOS genes (33). The key to producing the inactivation reaction in vitro was a mutant of *recA* named *tif* (thermal induction of filamentation), which, upon a temperature shift, simulates the effect of DNA damage: λ is induced, along with other damage-dependent cellular processes (e.g., filamentation). Although *tif* might have been a mutation that activates RecA in the absence of some unknown cellular signal, its property turned out later to be simply more activity to bind DNA than the wild type under limiting conditions, as shown by graduate student Eric Phizicky (41); to anticipate discoveries described below, we inferred that *tif* RecA is activated by some natural DNA that wild-type RecA does not bind sufficiently avidly. Our initial experiments depended upon a λ lysogen isolated by David Mount that had not only high-level constitutive activity of the *tif* RecA protein (due to a mutation in the cellular *lexA* gene, which represses *recA*) but also a mutant λ repressor that resisted this activity at lower temperatures and allowed the lysogen to survive, but then was inactivated efficiently at higher temperatures. We could demonstrate repressor cleavage at the high temperature in permeabilized cells of this lysogen, dependent upon addition of ATP; the energy source was added mostly by analogy to known energy-dependent processes of protein degradation in eukaryotes, which turned out to be quite incorrect. We then were able to detect the ATP-dependent *tif* RecA activity for λ repressor inactivation and cleavage in extracts (53), with helpful advice from Raymond Devoret about the filter-binding assay for λ repressor, and to purify RecA protein as the active factor to homogeneity (52). No other protein factor could be identified by exhaustive purification, leading to the conclusion that RecA provides the proteolytic activity. The most important discovery in the sequence (by Nancy Craig as a graduate student) was that single-stranded DNA (ssDNA) is necessary for the activity of RecA to promote repressor cleavage, and that a stoichiometric filament of RecA and DNA, stabilized by binding of ATP, is in fact the active element (8). This result led to the model that the inducing signal for repair of DNA damage is ssDNA elaborated by processes of replication and degradation, a model that is generalizable beyond bacterial DNA repair. Specifically, the prediction that LexA also would be inactivated by this pathway turned out to be correct. Graduate

student Mandana Sassanfar showed, partly confirming some previous work, that DNA replication is necessary for UV induction of λ; thus, the inducing signal is a consequence of replication through damaged DNA, which we suggested to be single-stranded gaps in the chromosome (56). The model was elaborated in a review article written with Raymond Devoret in *Lambda II*, Cold Spring Harbor Press (50).

But what kind of protease is RecA? It was discovered by John Little and David Mount at the University of Arizona that LexA cleavage occurs at a detectable rate at elevated pH without the addition of activated (DNA-bound) RecA, which also is true of λ repressor (32). Thus, the active site for proteolysis is in the LexA and λ repressors themselves, and not in RecA, so that in fact they self-cleave; however, in normal conditions this reaction requires repressor binding to the RecA/DNA filament. The RecA-ssDNA filament is an enzyme in the sense that it accelerates an otherwise thermodynamically favorable reaction, but clearly it is not the usual sort of protease. Most important, however, is that RecA is in fact the inducer, and that ssDNA is the agent that activates it.

Termination and Antitermination

Our major laboratory direction for the last decades was the mechanism of transcription antitermination and termination. Other critical discoveries had been made since discovery of Rho and proposal of the N model. John Richardson at Indiana University found that Rho is the product of the gene *suA*, mutation of which suppresses genetic polarity, the failure to express genes of an operon downstream of one containing a nonsense codon (43). This showed directly that polarity is due to transcription termination, not mRNA stability as had been thought. Consistently, it had been shown that N suppresses genetic polarity in operons transcribed under its influence (1, 11).

Polarity suppression provides a possible answer to the frequent question of why antitermination exists as a transcription control device at all, since negative regulation by repressors and positive regulation by activators would seem to suffice. A plausible answer for bacteriophage like λ derives from their mosaic character. Evolution of the λ family features rampant recombination as phage acquire new DNA that presumably favors survival in a defensive bacterial environment. But this would entail novel joints that might frequently disrupt coding regions and introduce nonsense codons; polarity suppression would drive transcription through any such regions and thus allow novel recombinants to survive.

As we worked on the λ Q protein, the λ N protein antiterminator was purified and characterized, mostly by Jack Greenblatt of the University of Toronto, and was shown definitively to be a transcription antiterminator (18). N works in a complex of cellular proteins (NusA, NusB, NusE, NusG), mostly identified originally by David Friedman, Costa Georgopoulos, and Max Gottesman through genetic studies (12). A striking climax of these studies is the cryo–electron microscopy (cryo-EM) structure at atomic resolution N in association with a transcription complex and all four cofactors, providing strong hints of how N causes antitermination (27).

From the beginning at Cornell we continued to work on Rho factor, showing that transcription pauses are components of the λ Rho termination sites I found at Harvard (30) and characterizing the length of sequence required for Rho function (20). However, the most critical discoveries in this period were that Rho is an RNA-dependent ATPase, by John Richardson (34), and that it acts as a helicase (5), by Terry Platt at University of Rochester (although "RNA translocase" might be more precise). Structural elucidation of Rho and Rho-RNA complexes by James Berger coalesced these discoveries into a precise structural model of Rho interaction with RNA (60). How and where Rho might interact with RNAP remain elusive, although the elongation factor NusG can be an important component of this interaction (31).

The λ Gene *Q* Antiterminator, Pausing and Antipausing

Most of our efforts in transcription involved purifying and demonstrating the antitermination activity of λ Q protein and studying the mechanism of intrinsic (RNA hairpin) termination. As an entirely nucleic acid–encoded device, intrinsic termination turned out to be better suited to understanding the mechanism of Q protein function.

In order to guide their purification, we assayed the activities of Q proteins of both λ and the lambdoid relative phage 82 using the Zubay coupled in vitro transcription-translation system. Initially we made extracts from induced lysogens, but soon we could clone the genes for overproduction, and also for a second useful scheme: Labeling with ^{35}S of proteins made under direction of a Q^{λ}-overproducing plasmid in the Zubay extract system yielded Q^{λ} as the majority labeled product, providing a radioactive marker to guide purification. The first homogeneous preparation of Q^{λ} allowed graduate student Beth Grayhack to demonstrate definitively its activity as an antiterminator in vitro (16).

A second discovery by Beth Grayhack presaged a major theme of antitermination mechanisms. The small terminated transcript (~200 nucleotides) on which the antitermination activity of Q^{λ} acts also features a strong transcription pause 16 nucleotides from the start site. Beth showed that addition of Q^{λ} to a purified in vitro reaction after the pause is established results in overcoming the pause, thus chasing RNAP into productive (and antiterminated) transcription (17). This established two important points. First, the paused structure, and likely not RNAP bound to the promoter, is the substrate upon which Q^{λ} engages RNAP. Second, the Q^{λ} modification of RNAP inhibits transcription pausing. Antipausing eventually was realized as a fundamental activity of an antiterminator that, because pauses underlie both Rho-dependent and intrinsic termination, could account for antitermination—although as described below, there is a separate aspect of antitermination by Q. Graduate student Xianjie Yang showed later that both Q^{λ} and Q^{82} suppress pausing during extensive transcription downstream of the Q engagement sites (66). The molecular basis of the antipausing activity is still being investigated.

Sigma Also Is an Elongation Factor: The σ^{70}-Dependent Pause

Probably the most unexpected discovery we made was that σ^{70}, the paradigm of initiation factors, also can act on elongation complexes, because it is required for the pause at nucleotide 16 of the late gene transcript that provides the substrate for Q^{λ} protein engagement (45). Through directed mutational analysis of the early transcribed region, we knew that mutation of nucleotide +2A or +6T (named in the nontemplate strand) prevents both the +16 pause and Q function. The very-slow-to-arrive realization was that these two nucleotides reiterate downstream the most important nucleotides of the ideal −10 promoter sequence to which σ^{70} region 2 binds, namely tAtaaT. Although σ^{70} is released from its attachment to the promoter upon initiation, it remains bound to core RNAP for some time and thus is available to engage the −10-like sequence that induces pausing. Graduate students Brian Ring and Bill Yarnell showed that the paused complex contains σ^{70} (45), even though graduate student Mark Kainz had shown earlier that the Q^{λ} paused complex is a fully formed elongation complex (26); this also is true of the Q^{82} and Q^{21} initial paused complexes. When added to elongation complexes in *trans* in sufficient concentration, σ^{70} can induce pauses at −10-like sequences further downstream (15, 36). Numerous bacterial promoters have σ^{70}-dependent pauses close to the initiation site, but no biological function of downstream σ^{70}-dependent pauses has yet been shown (21).

A by-product of this discovery derived from Brian's previous demonstration that the pause-inducing sequence acts in the nontemplate strand of the DNA template and not the template strand (44): The nontemplate strand also must be the site of base-specific recognition of the −10

sequence in the promoter by σ^{70} region 2, as we showed directly (46). Structural analysis of the open promoter complex fully confirms this result and shows in atomic detail how the recognition occurs (2, 70).

Much remaining progress on the nature of Q engagement at the pause site was made by Ann Hochschild and students at Harvard Medical School, some in collaboration with us. In particular, they localized Q^{λ} binding to the RNAP β subunit flap (10) and also demonstrated by mutational analysis that σ^{70} region 4 is displaced to a novel location by binding of Q^{λ} (37).

Mechanism of Intrinsic Termination

The intrinsic terminator consists of sequences that in RNA constitute a hairpin and a following segment rich in uridine residues, presumably important because the weak rU/dA hybrid should facilitate unwinding of the RNA end (49). It has a specific structure: The base of the hairpin has to be 9 nucleotides from the RNA end of the released transcript. Clearly, formation of the hairpin disrupts the normal elongation structure in some fashion; since RNA from 9 to 14 or so nucleotides behind the RNA end is held within the RNAP channel, the hairpin would penetrate the RNA channel. Bill Yarnell in the laboratory showed that the hairpin can be replaced by a DNA oligonucleotide that mimics the upstream half of the RNA hairpin stem (67). The span of such an oligonucleotide that has RNA release activity quite precisely matches the domain of an active hairpin; thus, the 5′ end of the oligo must anneal to the emerging transcript about 9 nucleotides behind the RNA end. Since a DNA oligo works, it is not the RNA hairpin per se, but instead the formation of duplex nucleic acid that is important to termination. Static complexes stopped at random positions are released by such oligonucleotides, even in the absence of terminal U-rich segments. Presumably this reflects the lack of any kinetic element in the oligo-mediated release; whereas a pause at the release end gives a measured interval for dissolution of the complex by a natural terminator, facilitated by the weak RNA/DNA hybrid, the static reaction works at its leisure.

The Q Modification of RNA Polymerase Stabilizes Static Transcription Complexes

Oligonucleotide-mediated RNA release as a model of intrinsic termination allowed demonstration of a fundamental property of the Q antitermination modification. I mentioned above the antipausing activity of Q, which does inhibit termination. Bill Yarnell showed that Q^{82} also stabilizes a static transcription complex against RNA release by the oligonucleotide (67). Thus, Q^{82} induces a structural modification of the transcription complex that inhibits activity of the oligonucleotide, and presumably of the natural terminator hairpin. Either this modification or antipausing would explain antitermination of an intrinsic terminator. This result was elaborated by graduate students Smita Shankar and Asma Hatoum, who demonstrated directly that Q^{82} produces a barrier to annealing of an oligonucleotide in the critical area where the hairpin of an intrinsic terminator forms, by showing that the Q^{82} modification prevents the oligonucleotide from forming an RNase H–sensitive structure with the emerging transcript (58). Rho termination function also must be inhibited by antipausing or the barrier, although it is not yet clear how.

Smita and Asma also uncovered a role of NusA protein, a transcription factor discovered in a search for cellular proteins required for antitermination activity of the N protein. Based on that prior discovery, and no genetic evidence for Q at all, we found early on that NusA strongly stimulates overall antitermination with Q^{λ}, although less with Q^{82} (65). Although Q^{82} does induce antipausing in the absence of NusA, it does not make the barrier observed in static complexes (58). At this writing, a detailed explication of these various elements and their functions is arising from cryo-EM-based analysis of Q-modified RNAP complexes. A striking structure of RNAP modified

by the Q protein of phage 21 reveals that it forms a torus around the RNA exit channel that would prevent formation of the termination hairpin (59, 68). It could be noted that these structures are descendants of the transformative first structure of bacterial RNAP by Seth Darst in 1999 (69), which ever since has informed all mechanistic thoughts about transcription.

Forward Translocation Model of Termination and RNA Release

How does an RNA hairpin cause dissociation of the otherwise notably stable elongation complex? Bill Yarnell in the laboratory proposed that formation of the hairpin initiates outside of the complex during a transcription pause and forces the enzyme forward without synthesis (forward translocation), thereby shortening and destabilizing the RNA/DNA hybrid, leading to dissociation (67). In this model, the energy of formation of the hairpin would compensate for and presumably exceed the energy lost by shortening of the hybrid, thereby driving the movement. Tom Santangelo in the laboratory provided evidence for the model (55). A test of the model by single-molecule analysis confirmed the prediction that forward force would promote termination with the terminator that Yarnell and Santangelo had used, which has a polyU-rich segment interrupted by two G/C base pairs (28). However, for the more common structure of an uninterrupted polyU segment, hairpin formation induces shearing (28), in which the RNA is simply pulled out by the developing hairpin and intermediate stages of RNA extraction are stabilized by repeated base pairing of the terminal segments through their homopolymeric structure. Structural analysis shows that a hairpin can partially penetrate the RNAP clamp as some protein conformational changes occur, so that essential protein movements must accompany these RNA movements; presumably these loosen the tight grip of enzyme on the nucleic acid structure.

The notion that forward translocation of RNAP could destabilize the complex and lead to RNA release was strengthened by studies of the transcription-repair-coupling factor Mfd, performed by graduate student Joo-Seop Park. This protein promotes excision repair of lesions like pyrimidine dimers that stop RNAP, by binding a stopped RNAP and recruiting the Uvr proteins to the site (57). It also releases RNA from the complex and thus is a type of termination factor, as shown originally by Selby & Sancar (57). Without a specific model in mind, we asked how Mfd would treat a stably backtracked (arrested) complex; could it release RNA from this structure with the RNA more entwined in the complex? Joo-Seop found that not only can Mfd release the backtracked complex but it also first reverses the backtracking, pushing RNAP forward to a position from which it can resume productive synthesis (38). It does so through its activity as an ATP-dependent DNA translocase, binding a specific site of RNAP and pushing it along the DNA. Thus, Mfd promotes forward translocation, and it very likely carries out RNA release by this same mechanism: Release is the consequence if the RNAP active center cannot elongate the RNA chain and continue substrate-driven translocation with the full RNA/DNA hybrid, but instead the complex is driven forward by Mfd without RNA synthesis. Rho also was shown to impel forward movement of RNAP as it acts; here the force presumably is imposed on the RNA as the Rho translocase activity pulls it out of the complex (39). Recall that evidence for forward translocation was found specifically for an intrinsic termination site that does not end in a pure homopolymeric sequence, a property also of Rho termination sites.

Complete Model of σ^{70}-Dependent Pausing

I regard a set of experiments done by the last graduate students, Jeremy Bird and Eric Strobel, as a gratifying final chapter of the laboratory's efforts. This work definitively characterizes the transcriptional movements that underlie the σ^{70}-dependent paused structure upon which Q protein acts. The primary realization is that the pause is bipartite: RNAP is restrained by σ^{70}-dependent

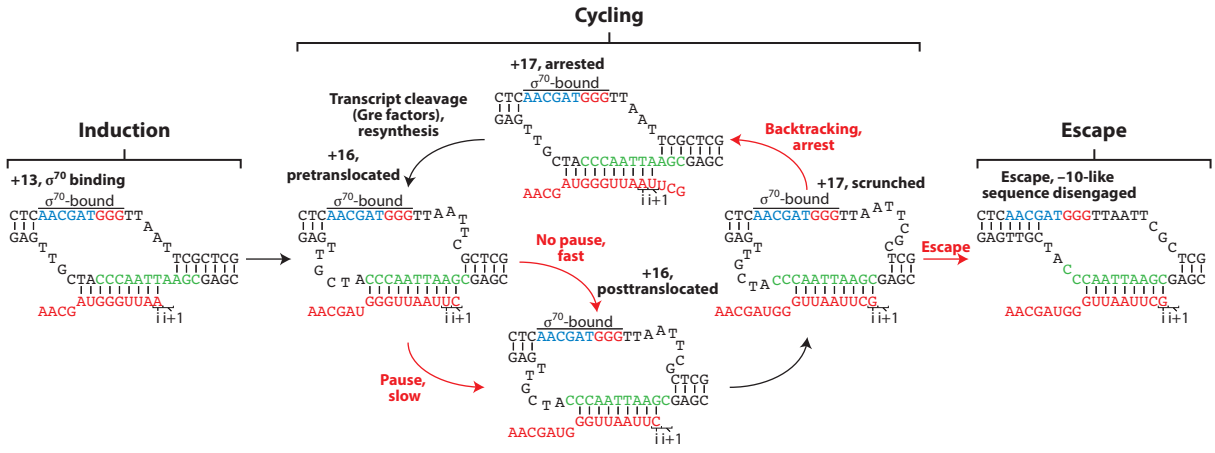


Figure 2

The σ^{70}-dependent pause cycle at the λ late gene promoter. When the active center of RNA polymerase arrives at +13, the σ^{70} nontemplate strand sequence AACGAT (shown in *blue*) is in the correct position to bind σ^{70}. Transcription continues with scrunching to +16, site of the actual pause induced by the elemental pause site (EPS), major elements of which are shown in green. Slow translocation to +17 results in either escape into productive elongation downstream or backtracking and arrest, followed by Gre-mediated cleavage back to +13; the cycle then continues. The overall pause lifetime depends upon the rate of translocation from +16 to +17, which is the intrinsic pause, and the fraction of +17 complexes that escape downstream rather than backtracking. Figure adapted with permission from Reference 61.

pauses binding a repeat of the −10 promoter sequence, but the actual pause site is determined exclusively by nucleic acid sequences that affect RNA/DNA hybrid stability and the interaction of nucleic acid elements with the core RNAP (3). The latter pause-inducing elements were discovered in a single-molecule analysis of sites of (brief) pausing of RNAP during elongation and have been named the elemental pause site (EPS) (22, 29, 62). In fact, the σ^{70}-dependent pause requires both σ^{70} binding and the EPS and is negligible if either is missing. We also realized that the pause is dynamic, consisting of cycles of pause, backtracking, Gre-mediated transcript end cleavage, and escape (**Figure 2**); a quantitative model that incorporated rates of these steps measured separately very accurately predicted the overall pause (61).

MOLECULAR BIOLOGY OF THE GENE

It was an unexpected opportunity to participate in writing the fourth edition of Jim Watson's *Molecular Biology of the Gene* (63), essentially the Bible of the early age of molecular biology. The first three editions were done entirely by Jim himself, but with the increased purview of molecular biology in the whole biological world the book needed expanding. Joan Steitz, Alan Weiner, Nancy Hopkins, and I each took several subjects, either updating or adding entirely new sections and chapters, under the constant watchful attention of Jim, of course. An attraction for me was knowing that laboriously honed sentences might be read by and important to many thousands of readers, in contrast to the ephemeral attention given to most prose of research papers.

EPILOGUE

My career ended with the ascent of genomics and its accompanying megadata analysis. This development has been transformative and can be greatly interesting to read about, but not my thing to do. In my era, if an experiment required statistics for its analysis, you needed to find a new

experiment—a saying I believe is attributed to some luminary I don't remember. One could imagine an experiment, discuss it with a graduate student, and see the result in a day or two; there might be 10 data points giving an unequivocal answer. For the student, as for me when I began, it was a largely individual endeavor, with logic, methodology, and experimental devices all plainly exposed and applied to some question of basic interest. No longer—prominent papers tend to be multigroup collaborations with tens of authors and distinct areas of expertise, often containing a plague of unmemorable acronyms. Likely no one understands everything. I was very lucky to arrive near the beginnings of molecular biology.

Other than those to which Chris contributed, essentially every experiment from Cornell upon which my career depended was done by a graduate student from the Cornell Graduate Field of Biochemistry, Molecular and Cell Biology, or, rarely, a different graduate field at Cornell; the few postdoctoral fellows in my laboratory were usually graduate students who stayed on for a year or two. This is certainly a tribute to a very successful graduate program, to strong support for research from Cornell University and from the College of Agriculture and Life Sciences to me in particular, and to the nurturing and collegial home provided by the original section and its successor Department of Molecular Biology and Genetics.

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