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An Introduction to Transcription and Gene Regulation

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Having been offered the opportunity to contribute one of the episodes of this series of personal history accounts, I have chosen to write about circumstances that led me to the start of the scientific path that I have followed for the past forty odd years. My account deals with a period of approximately six years and with events that I have written about recently (1); although I have been conscientious about avoiding overt self-plagiarism (at least the kind that is detected by computer programs), I also hope to have added some additional perspective.

To the extent that Reflections are accounts of the development of our science, they cover the same terrain as textbooks, albeit in a very different style and with somewhat different intent. To my mind, a potential source of wider interest (or at least different interest) of Reflections is that they provide a view of how greatly the ways of practicing our craft, of doing science, change within a single functional lifetime. That thought comes repeatedly to mind as I struggle to write this account. Having also read diligently what others have written for these occasions, I have been struck by how differently scientists view the circumstances of their scientific lives. I hope to have added to that variety.

The special role of the bacterial viruses in laying the foundations of molecular biology has been recounted at length in widely read histories of the subject (2–5). I came late to learning about phage, but they served as my introduction to enzymology, and they have been my companions for nearly fifty years. This brief personal account is about the role that phage played in my early biochemical education.

RNA Polymerase

When I joined the University of Chicago's Committee on Biophysics as an assistant professor in the fall of 1959, my mode of thinking about biological questions was very much that of a chemist, and my focus was narrowly on DNA. What had struck me when I first saw the proposed structure of the double helix was the way its purine and pyrimidine residues were sequestered in its core, whereas the charged sugar phosphate backbone was exposed to the surrounding milieu (somewhat micelle-like, but it was the analogy to the bimetallic spiral sensors of contemporary thermoregulators, with a different material on the outside (the sugar-phosphate helix) and the inside (the stacked base pairs), that I recall as having also come to mind). Finding that DNA denatures and collapses when water is substituted by ethanol (6) led to a wider exploration of DNA denaturation by other non-aqueous solvents. Ted Herskovits took up this subject for his Ph.D. thesis (at Yale) (7).

Early experiments at Chicago following these lines of thought included an exploration of the effects of "chaotropic" 1:1 electrolytes (in other words, the salts of the Hofmeister series) on the stability of DNA to thermal denaturation (8) and the effects of (intercalating) acridine dye-sensitized DNA photolysis by visible light on the thermal stability of DNA (9). Related experiments

intended to examine the extent to which disruption of base pairing by deamination (of A, G, and C) would affect thermal stability led serendipitously to the discovery that the deaminating mutagen nitrous acid additionally generates cross-links between complementary DNA strands (10) and that the introduction of a single cross-link into a DNA molecule renders its denaturation completely reversible (11).

When Sam Weiss came to the University of Chicago's Department of Biochemistry and Argonne Cancer Research Hospital, he turned his attention to the RNA synthesis problem. As he wrote in a personal history account (12), Sam initially decided not to work with bacterial extracts for fear of being confounded by the predominating activity of polynucleotide phosphorylase. He also decided to prepare the hypothesized ^{32}P-labeled nucleotide substrate [α-^{32}P]CTP exactly corresponding to the deoxyribonucleoside triphosphate substrates of DNA polymerase (13) and to demand dependence of ^{32}P incorporation on the presence of all four ribonucleoside *tri*phosphates. The specific attention to choosing his substrate and the labor of its preparation were quickly rewarded: CMP was incorporated into the nuclei-fraction from a rat liver homogenate in a process requiring all four ribonucleotides, inhibited by pyrophosphate but not orthophosphate, and distributing incorporated CMP residues next to A, U, and G nucleotides (14). All these properties distinguished the newly discovered activity from polynucleotide phosphorylase and implied the synthesis of polynucleotide (or, more specifically, of internucleotide linkages) of complex sequence. But was this process DNA-dependent? Finding that the incorporation of CMP was substantially inhibited by DNase I was indicative but not decisive; subsequent experiments with a lysed-nuclei preparation from rat liver showed an even greater sensitivity to DNase I but did not definitively resolve the issue (15). That required solubilizing the RNA polymerizing activity and freeing it sufficiently from endogenous DNA, something that eluded Sam Weiss, working with his eukaryotic (nuclear) RNA polymerase activity. (In fact, that obstacle was not overcome, and the eukaryotic RNA polymerases were not solubilized, for another five years or more (16, 17).)

By this time, Audrey Stevens and Jerry Hurwitz's group had independently discovered the comparable activity of bacterial extracts, which could be readily solubilized and substantially freed from endogenous nucleic acids (18, 19), allowing the requirement of its activity for added DNA to be directly and convincingly demonstrated (19) and, in the next step, to show that the relative rates of incorporation of the four ribonucleotides into synthesized RNA corresponded to the composition of added DNA (20).

Frustrated in his attempts to solubilize his RNA polymerase activity from animal sources, Sam Weiss already had turned to bacteria for his cell extracts (12), found them to be an order of magnitude more active, and also realized that they were readily freed of endogenous DNA so that the DNA dependence could be demonstrated (21). Shortly thereafter, Hurwitz and co-workers showed that, when supplied with alternating poly(dA-dT):poly(dA-dT), RNA polymerase incorporated alternating UMP and AMP residues into its synthesized product (22). Subsequently, Mas Nakamoto and Sam Weiss showed that the nearest neighbor frequencies of incorporated nucleotides corresponded, within acceptable margins of error, with the nearest neighbor nucleotide pair frequencies of the RNA synthesis-eliciting DNA (23).

It was the question of the relationship of DNA to the RNA product that brought me the opportunity to work with Sam. At that point, his work had established that the average nucleotide compositions of enzymatically synthesized RNA and synthesis-eliciting DNA were identical. The experiments extending this information to nearest neighbor frequencies were in prospect, but their outcome (23), to which I have just referred, was not yet known. At issue was the question of whether similarity of composition could be shown to reflect detailed correspondence of nucleotide sequence. Julius Marmur, Paul Doty, and co-workers had shown that reassociation of fully separated DNA strands occurs only when they originate from the same or closely related genomes (24, 25) and therefore provides a test of perfect or nearly complete complementarity of nucleotide sequence. Using CsCl density gradient equilibrium sedimentation, Ben Hall and Sol Spiegelman applied the same test to RNA ^{32}P-labeled after phage T2 infection of *Escherichia coli*: this RNA was able to reassociate with denatured T2 DNA but not with native T2 DNA or with denatured phage T5 or *E. coli* DNA (26). The experiment established that the synthesis, in phage T2-infected bacteria, of RNA with a nucleotide composition corresponding to that of the infecting phage DNA (27) extended to complementarity of nucleotide sequence.

We applied the same test to RNA that was synthesized by the bacterial (*Micrococcus luteus*) RNA polymerase in the presence of phage T2 DNA and found the parallel result: the *in vitro* synthesized RNA was able to associate with denatured T2 DNA (but not heterologous DNA of nearly equal GC content) and only under conditions allowing reassociation of complementary nucleic acid strands. Our experiments also yielded two additional insights: utilization of DNA for transcription did not irreversibly separate its strands, and the synthesized RNA did

not remain stably DNA-associated. These were the critical distinctions between the modes of action of RNA and DNA polymerases: the synthesis of RNA on a DNA template was conservative (with regard to template strands) rather than semiconservative, as it is in DNA replication. One finding of these experiments was surprising: in the presence of a sufficient quantity of T2 RNA, the buoyant density of all annealing DNA increased as a result of association with its complementary RNA, implying that both DNA strands were acting as template for RNA synthesis (28). In this case, transcription might be generating mutually complementary RNA strands. That this was indeed the case was shown next. The *in vitro* synthesized T2 RNA was seen to be self-complementary; under conditions appropriate for nucleic acid strand (re)association, it formed a distinctive material, with characteristics expected for double-stranded RNA of complex sequence, including resistance to degradation by RNase A and denaturation (melting) at a sharply defined temperature (29).[1]

Two experiments addressing the same question (whether only one strand of a gene serves as template for RNA synthesis) appeared to yield the same answer. Using the first highly purified and characterized RNA polymerase (from *E. coli*), Mike Chamberlin found transcription of the double-stranded (replicative intermediate) form of phage ΦX174 DNA yielding RNA with a nucleotide composition corresponding to that of its template and not of the strand that is packaged in the mature virion (or its complement) (30); Masaki and Marie Hayashi, working with Sol Spiegelman, obtained the same result (31). At the same time, an argument based on the pattern of phenotypic suppression of mutations in the phage T4 *rII* genes by 5-fluorouracil specified that the function of these two genes is mediated through the RNA product of only one DNA strand (32), that only one strand is informational. (The general conclusion of the argument turned out to be correct. Ironically, the argument itself, although elegant, much cited, and influential for that moment, was subsequently seen to have been flawed and invalid with regard to specific detail.)

Three questions were now posed. 1) Was this generally the case? 2) Was informational/functional asymmetry generated by initially asymmetric RNA production *in vivo* or by asymmetric processing of an initially symmetric RNA product derived from the use of both DNA strands as transcription templates? If RNA synthesis *in vivo* was found to be asymmetric, this would imply an ability to selectively utilize the transcription template and a failure of selectivity *in vitro*. 3) What might be the nature of that defect?

Tackling the first of these questions, the Hayashis and Spiegelman showed that ΦX174 RNA briefly pulse-labeled in phage-infected *E. coli* is complementary only to the DNA strand that is not packaged (33). Having found a way to preparatively separate the complementary strands of the large (~130-kilobase pair genome and ~140-kilobase pair DNA) 5-hydroxymethyluracil-containing *Bacillus subtilis* phage SP8, Julius Marmur and his co-worker were able to show that viral RNA labeled for several minutes of the phage multiplication cycle is almost entirely complementary to the DNA strand with the higher buoyant density (in CsCl, the difference resulting from an unequal partition of purine and pyrimidine residues to the strands) (34).

At Chicago, our attention turned to a clear plaque lytic mutant of the temperate *Bacillus megaterium* phage α. This phage (originally isolated from the waters of the Tiber) had been the subject of radiobiological experiments by a group of physicists and microbiologists in Rome; the results had suggested to them the possibility that its DNA might be single-stranded. Although this turned out not to be the case, the experiments establishing the double-stranded nature of α DNA showed its complementary strands to have different buoyant densities in CsCl (35), reflecting different base composition. The group in Rome set about the task of preparatively separating the DNA strands, and Franco Graziosi agreed that this material could be used to examine the question of template strand utilization *in vivo*. Marvin Stodolsky took on this project as part of his Ph.D. thesis research, and we were joined by Glauco Tocchini-Valentini (sent from Rome to encourage progress and steepen the learning curve), the start of a friendship that has enriched my own enjoyment of science for nearly fifty years. The experiments faced some hurdles (primarily the consequence of inexperience), but we learned how to get ^{32}P label into RNA of these cells and

[1] Sam Weiss' and my laboratories were situated in separate buildings, two blocks apart on Ellis Avenue: Sam's in the semibasement floor of the Argonne Cancer Research Hospital building and mine on the top floor of the Research Institutes building, across the street from Stagg Field and the stands of the football stadium that, less than twenty years before, had been the site of the first sustained nuclear chain reaction. Our initial experiments were done primarily in Sam's laboratory, where he had a hand-operated radiation monitor and counter, lacking a printer. Each sample from an experiment was loaded into the monitor's disk-shaped sample holder and rotated into position for counting, the count was recorded, and the sample holder was rotated again for removal of the just counted sample so that the next sample could be loaded, the outcome of the experiment developing serially and at a pace that allowed time for discussion and planning of the next experiment. I think nostalgically about the qualities of directness of those first experiments and of their rapidly rendered judgments, the combined product of our limited means, and, in my own case, an only schematic understanding that encouraged generalization.

extract their nucleic acids. The continuing synthesis of host cell RNA during phage infection called for an initial step of separating phage-specific from host cell-specific RNA (by hybridization to excess phage DNA, collection of DNA:RNA hybrid duplexes, dissociation of that product, and complete separation from DNA); preparative chromatographic separation of the complementary DNA strands also proved to be challenging, but it was possible (in Rome) periodically to separate substantially pure light (lower buoyant density in CsCl; purine-rich; designated L) DNA strands. This was verified by CsCl density centrifugation after prehybridization to confirm the absence of material corresponding to heavy strand or renatured DNA.[2] The L-strand fraction of α DNA was used as template for synthesis of its complementary (α_H) RNA *in vitro*, thus substituting for the unavailable heavy DNA strand; α_L DNA and α_H RNA sufficed for the desired examination of ^{32}P-labeled RNA extracted from bacteria late in the phage α infection cycle. The outcome of the analysis was unambiguous: the RNA from phage-infected cells was able to form RNase A-resistant duplexes with α_H RNA and with denatured α DNA but not with L-strand (α_L) DNA. As a control, the latter could be shown to form DNA:RNA hybrids with self-complementary RNA synthesized *in vitro*. The ^{32}P distribution among nucleotides of α RNA synthesized in the infected cell also corresponded nicely with the composition of L-strand α DNA (37).

At the Cold Spring Harbor Symposium at which these results were briefly presented by Glauco Tocchini-Valentini, Julius Marmur and Sol Spiegelman presented their comparable finding with phage SP8 and ΦX174 (38, 39). Ben Hall also presented work of his laboratory showing that a fraction of phage T2 DNA could not be made to form DNA:RNA hybrid duplexes with a mix of RNAs made throughout the phage multiplication cycle (40), whereas E. K. Bautz showed T4 RNA isolated from phage-infected *E. coli* to be unable to form RNA:RNA duplexes (41). Both reports contrasted with what had been found originally for the *in vitro* synthesized T2 "C-RNA" (28, 29), consistent with the conclusion, stated by Bautz, that the genetic information is transcribed *in vivo* from only one of the two DNA strands.[3]

The common emphasis on DNA strand-selective asymmetric synthesis of RNA *in vivo* returned attention to RNA polymerase and *in vitro* transcription. Asymmetric transcription signified the ability to select functionally appropriate DNA sequence, and it appeared from the outset that this selection must occur at initiation of transcription. What was the defect that kept *in vitro* transcription from accurately reflecting the cellular process?

Masaki and Marie Hayashi and Spiegelman turned their attention to this question. Starting with the Chamberlin-Berg method for purifying *E. coli* RNA polymerase (30), they separated two fractions of comparable and high specific activity and showed that one of these transcribed the double-stranded circular replication intermediate ("replicative form") DNA asymmetrically, with the non-packaged DNA strand serving as the transcription template (as they had already shown to be the case *in vivo*). Disrupting ΦX174 circular replicative form DNA (the DNA being small, it was necessary to use sonication for this purpose) destroyed the asymmetric quality of its transcription so that both DNA strands were now transcribed. The second RNA polymerase fraction also failed asymmetric transcription. In interpreting these findings, the Hayashis and Spiegelman fixed their sights on DNA continuity and circularity as critical for asymmetric transcription and even extended that speculation to all transcription. Failures of their second RNA polymerase fraction were attributed to its conceivable contamination with an endonuclease, disrupting the circular DNA template (45), in other words, to something unwanted present rather than something essential missing.[4] The discovery of the sigma factor five years later (46) makes it almost certain that the "other" RNA polymerase fraction, with its discrete pattern of chromatographic elution, had lost its σ subunit.

In the meantime, I had prepared crude extracts (the supernatant fraction from ribosome-pelleting ultracen-

[2] The apparently greater degree of difficulty of separating the strands of phage α relative to phage SP8 DNA may have been due to the different chromatographic properties of T- and 5-hydroxymethyluracil-containing DNA and the different distributions of purines and pyrimidines in the complementary strands of the respective phage. The difficulty of consistently preparing the chromatographic matrix, which consisted of methylated serum albumin adsorbed to kieselguhr, a diatomaceous earth composed principally of silica, also may have been a contributing factor (36).

[3] There are significant caveats attached to the latter experiments. The coding strands of T2 and T4 genes are not all co-linear; thus, finding T2 DNA "strands" incapable of hybridization with a mixture of RNAs made throughout the T2 multiplication cycle (40) implies considerable fragmentation of that T2 DNA. Formation of RNA:RNA duplexes by annealing is concentration-dependent; the failure to form RNA:RNA duplexes was recorded at unspecified RNA concentrations, a trivial detail, of course, except that the subsequently discovered synthesis of mutually complementary RNAs at different times of the phage T4 infectious cycle (42, 43) was missed. Five years on, A. Guha and W. Szybalski discovered how to separate DNA strands by allowing them to associate with poly(ribo)(U) (exploiting the asymmetric distribution of nucleotides between DNA strands). It was then a simple matter to show that RNA pulse-labeled at the earliest time after phage infection (T4 "immediate early" RNA) is entirely complementary to the T4 DNA strand with the lower affinity for poly(U) (44).

[4] DNA supercoiling and the topological properties of closed circular double-stranded DNA were not yet understood, so the possible distinction between the properties of closed and nicked circular DNAs as transcription templates was not made.

trifugation) from uninfected *B. megaterium* and had found to my astonishment and delight[5] that the RNA produced *in vitro* appeared to correspond in its strand selection to the natural transcription product of phage α-infected cells and that fidelity of the *in vitro* transcription process was manifested in the presence of all the components present in that cytoplasmic extract. This made an emphasis on DNA continuity and circularity (45), and its generalization as a principle governing all transcription, appear implausible. Shearing α DNA to predominantly one-half, one-quarter, and one-eighth length fragments (by repeatedly forcing through syringe needles of graded diameter), it was a straightforward matter to show that transcriptional fidelity of the crude bacterial extract was retained. Fidelity was lost (and both DNA strands were transcribed) when α DNA was denatured and restored when the separated strands were reassociated. Both results implied selection of specific DNA sites for initiation of transcription that required double-stranded DNA but not intact phage α chromosomes.[6]

Up to this point, success in asymmetric initiation site-selective transcription had been confined to the use of conjugate DNA and transcriptases. Crude ribosome supernatant and ammonium sulfate-precipitated fractions were easy to prepare, and asymmetry of transcription could be assessed by testing for (in)ability of the *in vitro* synthesized RNA to form RNase-resistant RNA:RNA duplexes. It was therefore a relatively simple matter to show heterologous template:enzyme combinations (α DNA with *E. coli* or *Pseudomonas fluorescens* extracts, as well as T2 DNA and a *B. megaterium* extract, for example) also yielding asymmetric transcription (47). That retention of function in heterologous systems can now be understood as reflecting the conservation of sigma, RNA polymerase, and bacterial promoter structure.

Phage

I can date the start of my fascination with bacteriophages to reading Mark Adams' book (48) when it first appeared in early 1959. Phage were an important part of the direction in which research was pointing, and Marvin Stodolsky had included some simple experiments in his thesis characterizing the phage α lytic multiplication cycle. I wanted to learn more, so I applied to take the 1963 Cold Spring Harbor phage course (Frank Stahl was in charge that year) but was not accepted, a fortunate circumstance because that summer turned out to be a busy and exciting time in the laboratory and because the rebuff, such as it was, focused my attention on the possibility of using a sabbatical to achieve the same ends. The appropriate family discussion having been held and Eduard Kellenberger, in Geneva, having responded positively to my inquiries, we sailed for Europe in the late summer of 1964. Regularly scheduled year-round transatlantic steamship service by competing French, British, Dutch, Norwegian, Italian, and United States companies was still in existence, and the circumstances of travel now emphasized the comfort and enjoyment of passengers, a big change from my first passage twenty years before, in the waning months of the war in Europe in a convoy with empty Liberty ships, to afternoon tea with music, which our 4-year-old son, Jonathan, especially enjoyed.

To prepare for work of the coming year, I also applied to take the new European version of the phage course. Instituting a course directly emulating the intense laboratory-based style of its Cold Spring Harbor model had been under discussion for several years in Europe not only for its didactic sake but as a way of integrating European science and reforming styles of scientific training regarded as petrified by outmoded tradition. For some, as is often the case in human affairs, these were discussions about what others should do. Fortunately, Franco Graziosi and Adriano Buzzati-Traverso, who were establishing the new International Laboratory of Genetics and Biophysics (LIGB) in Naples, saw such a course as directly related to the mission and style of their new institute and as a way of placing LIGB on the scientific map, and Eduard Kellenberger offered to organize the teaching. That the considerable logistic challenges of setting up the course at the new campus were mastered was certainly due to Franco Graziosi, and it had first been offered in the summer of 1963. With Eduard Kellenberger, my prospective host, and our recent collaborator, Franco Graziosi, in charge of the second offering of the course, admission was this time assured. The course itself was divided into segments dealing respectively with lytic and lysogenic phage segments, taught by Dick Epstein, Toinon (Antoinette) Bolle, Werner Arber, and Enrico Calef. Its style and pace were true to its Cold Spring Harbor model, but the difference between the genteel austerity of the Long Island North

[5] The first *in vitro* experiment with that crude cell fraction and phage α DNA was done without great expectation, but its RNA product was duly analyzed for hybridization with L-strand α DNA, on hand from the *in vivo* experiments. I recall loading the corresponding samples into the scintillation counter, going home, returning (more out of general habit than specific expectation) later that evening, and immediately realizing that I was looking at the apparent answer to the question that I had been chasing for nearly three years.

[6] It is likely that the contrasting result with ΦX174 DNA was contributed by artifacts: generation of "frayed" single-stranded DNA ends by sonication and initiation of transcription at these single-stranded ends; and the inclusion of Mn^{2+} in the transcription assay medium, possibly favoring this DNA end-initiated transcription.

Shore and the disorderly vibrancy and beauty of the Naples setting certainly inflected its separate character.

Basic training having been accomplished, I returned to Geneva to start research with two ideas in mind: 1) that my laboratory in Chicago would continue to function, pursuing transcription-related objectives and, with the help of a newly purchased tape recorder, that it would be a simple matter to stay in touch with the details of experiments there; 2) that a sabbatical should be used to learn and do something new. The first of these ideas proved to be an illusion, as I realized by the end of that fall; the second determined my initial project. I was interested in the problem of how DNA is packaged in the mature virus particle and thought that the process needed to involve DNA condensation as an initiating step. (The idea of the actual process, of an ATP-consuming molecular motor stuffing DNA into the preassembled phage head against progressively increasing and ultimately prodigious resistance, never occurred to me, and I do not recall it being discussed at the time.) Eduard Kellenberger drew my attention to a phage T4 mutant that produced a large proportion of defective particles with shorter heads packaging incomplete T4 genomes and suggested that I join Fred Eiserling in looking into it. The "petite" (*pt*) T4 mutation generated characteristically small plaques and mapped to the cluster of head-determining genes, close to gene 23, which encodes the major head protein. The production of phage particles with normal and isometric heads in cells infected with petite mutant phage could be scored (by Fred Eiserling) in the electron microscope, and this allowed complementation tests to be constructed. Thus, I happily spent the autumn months doing phage crosses, frequently together with Dick Epstein and Fred, who turned out to be tolerant of the sabbatical visitor's slower hands. However, the work itself was on a wrong track: the mapping and complementation analysis (done with only single *amber* mutants in each gene) indicated that the *pt* mutation was in a separate complementation group located next to gene 23. Later functional analysis in Fred Eiserling's new laboratory at the University of California, Los Angeles, did not contradict that conclusion (49), and Eduard Kellenberger suggested that the "new" gene might encode a phage head-determining morphogen. We were wrong. More detailed mapping subsequently showed the *pt* mutation to be located within the gene that encodes the major head protein and that certain missense mutants of this gene generate phage with giant as well as shortened heads. There is no separate master gene encoding a morphogen of phage heads.

In any event, work with the *pt* mutation and phage head morphogenesis appeared to be coming to a possible stopping point by the end of the year, and my thirst to work on something other than transcription was getting satisfied. At the same time, a laboratory in which biochemical experiments using radioactivity could be done conveniently was becoming available in the neighboring building, newly constructed to house Alfred Tissières' laboratory.

The discovery of the *amber* (translation-terminating) mutants had led to the monumental construction of the circular phage T4 genetic map and correlated structural analysis of the virus multiplication cycle (50). One of the striking findings of that work was the discovery of a new class of phage mutants that do not make any of the virus subassemblies (the head, tail, tail plate, and tail fibers) that appear at late times of the normal multiplication cycle. DNA replication was seen to be required to produce these "late" proteins (51). In addition, "maturation-defective" mutants in two genes (gene 33, one of the 52 genes constituting the original circular T4 map, and the recently identified gene 55) were found to replicate DNA essentially normally but make no virion parts. The question before us was whether mutants of these genes were also globally defective for making the mRNA associated with late functions, in other words, whether genes 55 and 33 might encode master regulators of the transcription program determining this developmental process.

The synthesis of different mRNA molecules at different times after T2 and T4 infection was first detected by Tamiko Kano-Sueoka and Sol Spiegelman as well as by Roman Khesin and M. F. Shemiakin. Soon thereafter, Ben Hall and Khesin, together with their respective co-workers, independently devised hybridization-competition analysis as a method for quantitatively characterizing the synthesis and accumulation of the products of this selective and time-dependent reading of the viral genome (52, 53). We turned to the simple method described by Agnar P. Nygaard and Hall to compare RNA synthesis in nonpermissive *E. coli* infected with wild-type and gene 55 and gene 33 *amber* mutant phage during the first minutes after infection, before the onset of DNA replication ("early" RNA) and later, when assembly of progeny virus particles is under way. The hybridization-competition method and associated RNA preparation and labeling were quickly mastered, and Toinon Bolle, Dick Epstein, and I were promptly rewarded with a clear answer: the synthesis of RNA normally made at late times of the infection cycle did not materialize in the absence of gene 33 or gene 55 function. The products of these two viral genes were evidently

required for transcription of the large segment of the phage T4 genome comprising the late genes (50).

Early experiments on transcription of T2 DNA with RNA polymerase from uninfected *E. coli* had indicated to Khesin and co-workers that the RNA produced *in vitro* resembled RNA made early in the phage multiplication cycle, before the onset of DNA replication, that is, early RNA (54). Similar experiments by Luria (55) and in Chicago (first reported in Ref. 56) confirmed that observation. That finding had led Khesin and co-workers to examine the transcription properties of extracts prepared from phage T2-infected *E. coli* collected late in the multiplication cycle, but transcription with these extracts failed to produce significant proportions of RNA that could be classified as late by hybridization-competition analysis.

I was skeptical of this finding. My prior experience with the apparent transcriptional fidelity of crude bacterial extracts (47) led me to expect that the functions of the gene 55 and gene 33 products might be manifested in a straightforward way in *in vitro* transcription experiments.[7] Accordingly, essentially repeating Khesin's prior work, I prepared a crude extract from wild-type phage T4-infected cells, used that material to transcribe T4 DNA, and analyzed the labeled RNA product by hybridization-competition against RNA extracted from wild-type phage-infected bacteria at early and late times of the multiplication cycle. My results merely confirmed what Khesin's group already had seen: I also failed to detect the synthesis of late RNA in anything like the proportions in which it was being produced in the phage-infected cells from which the extracts were made. The *in vitro* experiments were put aside for the remainder of the sabbatical year, and attention was focused instead on the analysis of RNA synthesis in the viral replication cycle (59). The determination to understand the mechanisms regulating transcription of the T4 late genes set the paths of my research upon returning to Chicago. I never lost my fascination with the question, although it took more than twenty years to get to the start point of the experiments that would provide the long awaited answers and another five years to lay the groundwork of the current understanding of this chapter in the enzymology of gene regulation. I have written elsewhere about this pursuit (1) and what I currently understand about the topic (60).

[7] There was little doubt in my untutored mind that transcription of the T4 late genes would prove to be under the positive control of an activator and that *in vitro* transcription experiments were the direct way to establish this by answering a simple question: is the gene in question transcribed by RNA polymerase using bare DNA as template? For bacterial genes, around which the original operon model had been formulated and around which arguments regarding the existence of positive control of the expression of genes still swirled (57), the means to answer the question directly did not yet exist. T4, with its large block of coordinately regulated genes, allowed the question to be addressed with methods that were at hand (52, 58). In fact, a negative answer to the question, as posed above, did not entirely close the door on negative regulation. It was conceivable that repression of the late genes might be built (by mechanisms unknown) into the T4 genome as it was being packaged in the mature progeny virus particle and that DNA replication was required to reverse this repression. That possibility had occurred to Roman Khesin as a way of reconciling his findings (54) with the postulated universality of negative regulation of the original operon model.

Debts

I am conscious of having written an account of science recalled as done in simpler times. Nostalgia for an only selectively recalled or nonexistent past is a common part of current public as well as private discourse. There is quite enough of it; another aliquot is not essential. Nevertheless, I do believe that the times about which I write really were, in important aspects, less daunting for a scientist at the beginning of a career than they are today. I was in a great deal of doubt when I decided to go to graduate school, as 20-year-olds commonly are, but the prospect of being able to practice the profession for which I was preparing was not the subject of those doubts. The National Institutes of Health (NIH) started its predoctoral and postdoctoral fellowship programs while I was in graduate school, and I was able to hold one of each. When I wrote my first grant application to the NIH five years later, the concept of disapproving applications for research support on grounds of being "too ambitious" and providing "insufficient preliminary data" had not yet been invented. In fact, I do not recall those now common terms of disparagement having been invoked when I served on an NIH study section several years later; they are a perverse modern development. In a period of less qualified public enthusiasm for science, the relationship between the resources available for publicly supported research and the size of the research community favored applicants for those research funds.

Two consequences followed. First, it was easier to measure success in science by what was being attempted and achieved rather than how much money was being collected. (Of course, the latter tendency is longstanding, if not eternal.) More importantly, the element of reversibility of fortune, which I regard as an essential component of a robustly organized scientific endeavor, was still retained: the defeat of a failed idea or project was no less profoundly discouraging than it must always be, but it was not the financial end of the line that is the most likely outcome today, especially for the young scientist at the beginning of a research career. The principal consequence, to my mind, was a less cautious spirit. Conservatism was also less the norm because caution weighs less heavily on the innocent:

molecular biology was not the massive body of knowledge and technologies that it is today.

I have accumulated many personal debts as a scientist. Sam Weiss and Dick Epstein introduced me to transcription, phage, and gene regulation. Ray Zirkle and Bill Bloom brought me and a small group of young scientists to the Committee on Biophysics at the University of Chicago and then watched over us benevolently as we went about our work. They and we (Bob Haselkorn, Ed Taylor, Bob Uretz, and Bob Haynes) managed to create a climate of excitement about science in which ideas were freely proposed and energetically dissected. Glauco Tocchini-Valentini came to Chicago to work in my laboratory at an exciting time. Our friendship and late night discussions have enriched the ensuing years of my life as a scientist. I have encountered admirable scientists whose continuing friendships I treasure. The NIH has been the financial mainstay of my research. One of its research career development awards allowed me to spend a critical sabbatical year in Geneva with Dick Epstein, and my research has been supported primarily by the extramural programs of its constituent institutes.

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REFERENCES

1. Geiduschek, E. P. (2009) *Annu. Rev. Biochem.* **78,** 1–28
2. Cairns, J., Stent, G. S., and Watson, J. D. (eds) (1966) *Phage and the Origins of Molecular Biology*, Cold Spring Harbor Laboratory Press, New York
3. Jacob, F. (1987) *La Statue Interieure,* Editions Odile Jacob, Paris
4. Judson, H. F. (1979) *The Eighth Day of Creation*, Simon & Schuster, New York
5. Stent, G. S. (1963) *Molecular Biology of Bacterial Viruses*, W. H. Freeman, San Francisco
6. Geiduschek, E. P., and Gray, I. (1956) *J. Am. Chem. Soc.* **78,** 879–880
7. Herskovits, T. T., Singer, S. J., and Geiduschek, E. P. (1961) *Arch Biochem. Biophys.* **94,** 99–114
8. Hamaguchi, K., and Geiduschek, E. P. (1962) *J. Am. Chem. Soc.* **84,** 1329–1338
9. Freifelder, D., Davison, P. F., and Geiduschek, E. P. (1961) *Biophys. J.* **1,** 389–400
10. Geiduschek, E. P. (1961) *Proc. Natl. Acad. Sci. U.S.A.* **47,** 950–955
11. Becker, E. F., Jr., Zimmerman, B. K., and Geiduschek, E. P. (1964) *J. Mol. Biol.* **8,** 377–391
12. Weiss, S. B. (1976) in *RNA Polymerase* (Losick, R., and Chamberlain, M., eds) pp. 3–13, Cold Spring Harbor Press, New York
13. Josse, J., Kaiser, A. D., and Kornberg, A. (1961) *J. Biol. Chem.* **236,** 864–875
14. Weiss, S. B., and Gladstone, L. (1959) *J. Am. Chem. Soc.* **81,** 4118
15. Weiss, S. B. (1960) *Proc. Natl. Acad. Sci. U.S.A.* **46,** 1020–1030
16. Furth, J. J., and Ho, P. (1965) *J. Biol. Chem.* **240,** 2602–2606
17. Liao, S., Sagher, D., and Fang, S. M. (1968) *Nature* **220,** 1336–1337
18. Stevens, A. (1960) *Biochem. Biophys. Res. Commun.* **3,** 92–95
19. Hurwitz, J., Bresler, A., and Diringer, R. (1960) *Biochem. Biophys. Res. Commun.* **3,** 15–19
20. Furth, J. J., Hurwitz, J., and Goldmann, M. (1961) *Biochem. Biophys. Res. Commun.* **4,** 362–367
21. Weiss, S. B., and Nakamoto, T. (1961) *Proc. Natl. Acad. Sci. U.S.A.* **47,** 694–697
22. Furth, J. J., Hurwitz, J., and Goldmann, M. (1961) *Biochem. Biophys. Res. Commun.* **4,** 431–435
23. Weiss, S. B., and Nakamoto, T. (1961) *Proc. Natl. Acad. Sci. U.S.A.* **47,** 1400–1405
24. Marmur, J., and Lane, D. (1960) *Proc. Natl. Acad. Sci. U.S.A.* **46,** 453–461
25. Doty, P., Marmur, J., Eigner, J., and Schildkraut, C. (1960) *Proc. Natl. Acad. Sci. U.S.A.* **46,** 461–476
26. Hall, B. D., and Spiegelman, S. (1961) *Proc. Natl. Acad. Sci. U.S.A.* **47,** 137–163
27. Volkin, E., and Astrachan, L. (1956) *Virology* **2,** 149–161
28. Geiduschek, E. P., Nakamoto, T., and Weiss, S. B. (1961) *Proc. Natl. Acad. Sci. U.S.A.* **47,** 1405–1415
29. Geiduschek, E. P., Moohr, J. W., and Weiss, S. B. (1962) *Proc. Natl. Acad. Sci. U.S.A.* **48,** 1078–1086
30. Chamberlin, M., and Berg, P. (1962) *Proc. Natl. Acad. Sci. U.S.A.* **48,** 81–94
31. Hayashi, M., Hayashi, M. N., and Spiegelman, S. (1963) *Science* **140,** 1313–1316
32. Champe, S. P., and Benzer, S. (1962) *Proc. Natl. Acad. Sci. U.S.A.* **48,** 532–546
33. Hayashi, M., Hayashi, M. N., and Spiegelman, S. (1963) *Proc. Natl. Acad. Sci. U.S.A.* **50,** 664–672
34. Marmur, J., and Greenspan, C. M. (1963) *Science* **142,** 387–389
35. Cordes, S., Epstein, H. T., and Marmur, J. (1961) *Nature* **191,** 1097–1098
36. Aurisicchio, S., Dore, E., Frontali, C., Gaeta, F., and Toschi, G. (1964) *Biochim. Biophys. Acta.* **80,** 514–516
37. Tocchini-Valentini, G. P., Stodolsky, M., Aurisicchio, A., Sarnat, M., Graziosi, F., Weiss, S. B., and Geiduschek, E. P. (1963) *Proc. Natl. Acad. Sci. U.S.A.* **50,** 935–942
38. Marmur, J., Greenspan, C., Palecek, E., Kahan, F., Levine, J., and Mandel, M. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **XXVIII,** 191–199
39. Spiegelman, S., and Hayashi, M. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **XXVIII,** 161–181
40. Hall, B., Green, M., Nygaard, A., and Boezi, J. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **XXVIII,** 201–203
41. Bautz, E. K. (1963) *Cold Spring Harbor Symp. Quant Biol.* **XXVIII,** 205–210
42. Jurale, C., Kates, J. R., and Colby, C. (1970) *Nature* **226,** 1027–1029
43. Geiduschek, E. P., and Grau, O. (1969) in *RNA Polymerase and Transcription: Proceedings of the First Lepetit Symposium* (Silvestri, L., ed) pp. 190–203, North-Holland Publishing Co., Amsterdam
44. Guha, A., and Szybalski, W. (1968) *Virology* **34,** 608–616
45. Hayashi, M., Hayashi, M. N., and Spiegelman, S. (1964) *Proc. Natl. Acad. Sci. U.S.A.* **51,** 351–359
46. Burgess, R. R., Travers, A. A., Dunn, J. J., and Bautz, E. K. (1969) *Nature* **221,** 43–46
47. Colvill, A. J., Kanner, L. C., Tocchini-Valentini, G. P., Sarnat, M. T., and Geiduschek, E. P. (1965) *Proc. Natl. Acad. Sci. U.S.A.* **53,** 1140–1147
48. Adams, M. H. (1959) *Bacteriophages*, Wiely Interscience, New York
49. Eiserling, F. A., Geiduschek, E. P., Epstein, R. H., and Metter, E. J. (1970) *J. Virol.* **6,** 865–876
50. Epstein, R. H., Bolle, A., Steinberg, C. M., Kellenberger, E., Boy de la Tour, E., Chevalley, R., Edgar, R. S., Susman, M., Denhardt, G. H., and Lielausis, A. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **XXVIII,** 375–394
51. Wiberg, J. S., Dirksen, M. L., Epstein, R. H., Luria, S. E., and Buchanan, J. M. (1962) *Proc. Natl. Acad. Sci. U.S.A.* **48,** 293–302
52. Khesin, R. B., Gorlenko, Z. M., Shemiakin, M. F., Bass, I. A., and Prozorov, A. A. (1963) *Biokhimiia* **28,** 1070–1086
53. Nygaard, A. P., and Hall, B. D. (1964) *J. Mol. Biol.* **9,** 125–142
54. Khesin, R. B., Shemiakin, M. F., Gorlenko, Z. M., Bogdanova, S. L., and Afanas'eva, T. P. (1962) *Biokhimiia* **27,** 1092–1105
55. Luria, S. E. (1965) *Biochem. Biophys. Res. Commun.* **18,** 735–742
56. Geiduschek, E. P. (1965) *Bull. Soc. Chim. Biol.* **47,** 1571–1577
57. Epstein, W., and Beckwith, J. R. (1968) *Annu. Rev. Biochem.* **37,** 411–436
58. Hall, B. D., Nygaard, A. P., and Green, M. H. (1964) *J. Mol. Biol.* **9,** 143–153
59. Bolle, A., Epstein, R. H., Salser, W., and Geiduschek, E. P. (1968) *J. Mol. Biol.* **33,** 339–362
60. Geiduschek, E. P., and Kassavetis, G. A. (2010) *Virol. J.*, in press