Joys and Surprises of a Career Studying Eukaryotic Gene Expression

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In this Reflections, I review a few early and very lucky events that gave me a running start for the rest of a long and wonderfully enjoyable career. For the main part, a discussion is provided of what I recall as the main illuminating results that my many dozens of students and postdoctoral fellows (approximately 140 in all) provided to our biochemical/molecular biological world.

A n invitation to write “Reflections” for the Journal of Biological Chemistry makes one thing certain: I have been around in the “science biz” for quite some time, in fact since the 1950s. If ruminations of senior citizens are to be of any use, the recollections must be interesting enough to the knowledgeable middle-agers (say up to 60, or is it now 70?) and then to provide an illuminating story or two about getting started and then changing focus (maybe more than once), especially to the postdoctoral fellows who are ready to take the plunge. Graduate students these days have to swim in a sea virtually turgid with the daily avalanche of new information and may be momentarily too overwhelmed to listen to the aging, but perhaps they also may need to, or at least wish to, listen to the reflections of the older set. I firmly believe how we learned what we know can provide useful guidance for how and what a newcomer will learn.

Getting Started

Tales of success or failure in life often include reference to the fact that an individual is “not responsible for choosing her/his parents.” The inference being that luck, good or bad, is determinative. There is a parallel here with curious, aspiring young scientists and their mentors: their “scientific parents.” My luck in this regard was truly extraordinary.

Medical school in 1951 was my ticket out of the racist South, Columbus, Mississippi, to be exact, where I grew up. I narrowly avoided (courtesy of a last minute $900 loan from a bank president’s widow) accepting a $5000 four-year state-sponsored scholarship, the price of which was the obligation of five years of general practice in rural Mississippi. Instead, I landed at Washington University School of Medicine in St. Louis, where my mother’s oldest sister lived and which at the time had two major league baseball teams, a great attraction. The medical school was one of the earliest of what were coming to be called “science-based” medical schools, second only to Johns Hopkins in having a “full-time” (salaried) academic faculty, both preclinical and clinical.

In our second year, the redoubtable Arthur Kornberg had been appointed but had not yet arrived to assume the chairmanship of the Department of Microbiology. We were therefore farmed out for our laboratory course to any willing faculty member who had some familiarity with microbiology. Robert J. Glaser, an assistant professor of medicine who studied streptococcal disease, took me in. I learned bacteriological techniques and how to infect mice and not myself
with streptococci. Type 12 Group A streptococci had just been indicted as the culprit that caused most glomerulonephritis (reviewed in Ref. 1). By the end of the year, I had at least learned what dose, delivered intraperitoneally, allowed >50% of type 12-injected mice to survive, although there was no indication that they had any specific defect in their kidneys. The most significant outcome, at least for me, is that I discovered I loved doing the experiments.

Another student, Steve Morse, a transfer from Albany Medical College, joined Glaser’s laboratory at the end of the second year. Steve had had rheumatic fever and had sustained heart valve damage, a common aftermath of streptococcal infections. Together, we then undertook, using a variety of Group A streptococcal serological types, to give rabbits “strep throats.” We had learned from reading animal anatomy books in the medical school library that rabbits have submucosal patches of lymphocytes in the pharynx: “tonsils” (2). In fact, some of the repeatedly infected rabbits did develop reactive nodules in the ventricular heart muscle that somewhat resembled Aschoff bodies, characteristic of rheumatic fever according to our pathologist collaborator, Prof. Wilbur Thomas (3).

These results suggested that, in the absence of white blood cells, streptococcal infections persisted in vivo. My efforts, simple as they were, resulted in a paper in the Journal of Clinical Investigation, a prestigious medical journal then and now (4). This experience showed, if nothing else, a certain industriousness that earned me a 1954 interview (arranged by the saintly Carl V. Moore, by all odds the most popular professor of medicine at Washington U) with Harry Eagle, a famous scientist at the National Institutes of Health (NIH) who had spent quite a few years during and after World War II studying penicillin. Eagle accepted me not for 1955, the year I graduated, but only after a year of internship, which I took at Barnes Hospital, the teaching hospital at Washington University.

By the time I arrived in Bethesda in July 1956, Eagle (to my everlasting benefit) had turned his attention entirely to his new interest: growing animal cells in culture and proving the necessary nutrients that allowed successful continuous cell growth (5). His famous defined medium, Eagle’s medium, is still widely used throughout the world. Eagle was deeply involved in cell metabolism involving amino acids and especially in protein turnover in human cells (6). He and a colleague, the eminent virologist Karl Habel, had done one experiment showing that maximal production of poliovirus in HeLa cells required the same amino acids as for cell growth (7). However, Eagle was not interested in further virus work and suggested to me that I take up the virus project. He assigned me to a two-person laboratory,
on the other side of which was Robert (Bob) I. DeMars, Ph.D. (who was, as was I, at NIH at least partly to avoid the still active Korean War draft). Bob was S. E. (Salva) Luria’s third graduate student (James (Jim) Watson was the first). He handed me Luria’s General Virology, the first textbook ever written exclusively about viruses. Bob instructed me to read it, and he would teach me virology. I did, and he did.

Experiments on poliovirus followed, including developing the first poliovirus plaque assay in HeLa cells, establishing a one-step growth curve, and showing that every cell in a culture could act as an infectious center, all basic tenets of successful lytic bacteriophage experiments, which was the model Bob trained me to follow (8).

Leon Levintow, an experienced biochemist in the Eagle group, and I together developed a simple poliovirus purification scheme (9, 10) using cesium chloride equilibrium sedimentation gradients, a suggestion dropped by Jerome Vinograd (11), a friend of Eagle’s, on a visit to Eagle’s laboratory. Using radioactive amino acids (and later adenosine) added during infection at various times and then purifying the poliovirus at the conclusion of virus production, we established the time course of RNA and protein synthesis of the constituents of the purified virus (9, 10). Retrospectively (no surprise now), the RNA was formed ~2 h or so before the protein. How the RNA was involved in somehow directing virus protein synthesis was the obvious question these experiments raised but, of course, did not answer.

Bob was satisfied with the progress so far but demanded that I now learn some genetics. I had to acknowledge this was a glaring hole in my training, already recognized by me as inadequate in a variety of ways. In 1958, Bob advised me to apply for a fellowship in Paris with François Jacob.

I did apply to the Service de Physiologie Microbienne, headed by André Lwoff, who was father of modern lyogenic phage biology and who had become interested in animal virology. Encouraged by Bob, I was bold enough to say right off that I hoped to train with François Jacob, who was still at the time in Lwoff’s service. In late spring or early summer of 1959, I received a letter of acceptance from Lwoff to spend a year in his group working with Jacob beginning in September 1960.

Another aspect of scientific life had begun to dawn during the spring of 1959. I had met Jim Watson while he was visiting Bob, and Jim invited me to Harvard University to give a talk probably because his consuming interest, having conquered the structure of DNA, had become RNA (13). He proposed to Carroll Williams, the Chairman of the Biological Laboratories, that I be considered for an assistant professor appointment. What exactly happened I do not know (and Jim does not remember), but I received a nice note from Williams that he appreciated my visit but he distinctly did not go down the road of further engagement. Somehow (I do not know how), Salva Luria, Jim’s and Bob’s mentor, learned of me and contacted me. Salva was moving to the Massachusetts Institute of Technology (MIT) from Illinois to set up a microbiology group within the Department of Biology. I met him, and he offered me a job as an assistant professor. I told him that I very much wanted and felt I needed to go to Paris for my year with Jacob, and in his seignorial style, he said he would work out a salary for me in Paris from MIT funds (I think the money actually came from his National Science Foundation training grant), and so I accepted my first independent job to begin in June 1961.

So, for those readers who pay attention to dates, my arrival in Paris in late August 1960 coincided with the time a small segment of the biological world had just realized that the basis for bacterial metabolic switches, or for formation of an invading DNA-containing bacteriophage, was the necessity for a short-lived carrier of genetic information: a “messenger RNA.” After much brilliant bacterial and phage genetics largely by Jacob and Jacques Monod (14), Sydney Brenner, Jacob, and Matt Meselson had detected such molecules in the spring of 1960 (15). Also a group of workers in Jim’s group had produced evidence using very short pulses of radioactive uracil that suggested a special bacterial mRNA fraction (16). As I have described before (17), here I was a neophyte scientist who had done some experiments on poliovirus RNA and was interested in how polio worked its will on human HeLa cells, and I had landed in the magic kingdom that had the answer to how RNA could pull off such tricks.

I spent an enormously informative period of 10 months trying to drink in all of the intellectual ferment at the Pasteur Institute that was swirling around the control of gene expression by controlling mRNA levels. In the laboratory, I did some simple experiments showing that the extent of phage growth and the rate of β-galactosidase formation in ribosome-deficient cells were dependent on the number of ribosomes that were present in the cell. These experiments were described by Jacob at the 1961 Cold Spring Harbor meeting but were never finished and published (18).

So, to summarize my great good luck so far: first, I went to Washington University and worked in Glaser’s laboratory with penicillin, thereby earning the attention of Harry Eagle, who had, by the time I arrived at NIH, turned
entirely to mammalian cells in culture, opening up a new era in mammalian cell biology. Then, Eagle suggested that I study animal viruses in cultured cells and assigned me to a laboratory with Bob, who taught me some virology, which in turn earned me an invitation to go to Paris to study with Jacob at precisely the right moment in the history of molecular biology when attention turned to mRNA and its regulation. In addition, Bob or Jim had spoken to Salva, who gave me my first job at MIT. I contend that this is about as lucky as a youngster ever gets.

**Studying Eukaryotic RNA: The MIT Years (1961–1964)**

I headed home from Paris to start my first independent laboratory with the express aim of exploring mRNA in animal cells grown in culture. Animal cells were known, of course, to have an envelope surrounding the nucleus, the location of chromosomes and DNA. By autoradiography, $^3$H-labeled ribonucleosides were seen to be incorporated first into RNA in the nucleus, whereas basophilic dyes that stained RNA showed that the vast majority of RNA was actually cytoplasmic. Moreover, it took some hours for $^3$H-labeled ribonucleosides to label the cytoplasm heavily. Brief labeling of proteins with amino acids occurred mainly in the cytoplasm. So where, if it existed, would we be able to identify mRNA? Henry Harris in England, who inveighed against the mRNA idea, proposed that the rapidly labeled nuclear RNA was not moved to the cytoplasm, finally suggesting that functional RNA might even be formed in the cytoplasm (19, 20).

After arriving in Boston in June 1961, I organized the supplies and equipment in the new laboratory on the eighth floor of the Dorrance Building (named for the Campbell’s Soup family). We made our own Eagle’s medium from stock solutions, as was the custom at NIH, and I got HeLa cells growing in suspension (and on glass plates as a backup) and prepared to study incorporation of radioisotopes into RNA in growing and poliovirus-infected cells to search for messenger RNA. At NIH, following the lead of Alfred Gierer and Gerhard Schramm (21), who first showed that phenol extraction of tobacco mosaic virus yielded infectious RNA, I had extracted infectious poliovirus RNA from both infected cells and viruses (9). (There were obviously intact 7-kb molecules because a single base scission rendered the viral RNAs noninfectious (22).) I planned that this basic procedure would be folded into the mRNA search in cells.

My first postdoctoral fellow, Klaus Scherrer, a Swiss-trained chemist, crossed the Charles River from a Harvard laboratory to join me in starting RNA work in animal cells. (A detailed account of our several years at MIT is given elsewhere (pages 151–162 in Ref. 17).) A summary of the early findings includes the following. 1) We established a technique employing hot phenol (60 °C) and SDS that yielded total cellular RNA, DNA-free, which included all continuously labeled RNAs and, most importantly, the great majority of rapidly labeled RNA. 2) Sedimentation analysis of continuously labeled material revealed two RNAs slightly larger than bacterial ribosomal RNA that were likely HeLa cell ribosomal RNA. However, the most rapidly labeled RNA delivered a big surprise (23, 24). These briefly labeled RNA molecules ranged from 1–2 kb long to many molecules very much larger than polio RNA (7 kb). After ~15–20 min of labeling, discrete large molecules (45 S, ~14 kb; and 32 S, ~6.7 kb) dominated the sedimentation pattern, and these proved to be pre-rRNA (25). 3) Sheldon Penman, a physicist disaffected with the drift in experimental physics to only very large collaborative teams, joined us in the fall of 1962. We followed the lead of Jon Warner, a graduate student who worked with me for a few months on polio RNA association with *Escherichia coli* ribosomes. (Jon and I did get some *in vitro* poliovirus RNA-dependent amino acid incorporation into structures larger than single ribosomes (26).) Jon returned to Alex Rich’s laboratory on the seventh floor and, together with the postdoctoral fellow Paul Knopf, discovered polyribosomes in reticulocytes (Ref. 27; recounted on p. 158 in Ref. 17 and in Ref. 28).

Back on the eighth floor, we found HeLa cell polyribosomes that exhibited a range of sizes, from those similar to reticulocytes to some with two or three times as many ribosomes as the average of five in reticulocytes (29, 30). Polio-infected cells lost their pre-existing polyribosomes, which, by 3 h of infection, were replaced by giant poliovirus polyribosomes containing as many as fifty ribosomes. It seemed clear that, in polyribosomes, we had our hands on isolated cytoplasmic structures that contained mRNA.

The HeLa cell polyribosomes from cells labeled with RNA precursors for only 30–40 min allowed detection of an RNA that averaged ~1500 nucleotides in length using the unlabeled large and small ribosomal RNAs (5000 and 1800 nucleotides) as markers. Most important, the $^{32}$P-labeled polysomal RNA had an average base composition of ~45% G + C, similar to the total HeLa DNA base composition, whereas the rRNA and pre-rRNA molecules were ~68% G + C (29). The issue was joined very early: was mRNA processed from the large, heterogeneous, rapidly labeled nuclear RNA (hnRNA), as were the ribosomal 28 S and 18 S RNAs from the 45 S pre-rRNA?
Albert Einstein College of Medicine (1964–1968)

Our group moved to the Albert Einstein College of Medicine (AECOM) in June 1964. A full professorship and 250% increase in salary could not be refused when I had a wife and three sons to support. In addition, Harry Eagle had moved to AECOM and had recruited more scientists working on cultured cells and animal viruses, such as Matt Scharff, Jake Maizel, and Bill Joklik. However, my appointment was in the Department of Biochemistry, headed by Eagle’s friend, the eminent biochemist Abe White. My first AECOM graduate student, Deborah Bernhardt (Mowshowitz), soon showed that RNA processing was not limited to pre-rRNA; a pre-tRNA also existed. RNA processing was up in the air (31, 32).

My chief interest was to pursue the possibility of processing the large nonribosomal nuclear RNA (hnRNA for heterogeneous nuclear RNA) to yield mRNA. RNA:DNA hybridization had been developed, but the simple expedient of showing that specific hnRNA sequences could compete with the hybridization of total mRNA sequences was confounded by the existence of sequences (described first by Roy Britten with David Kohne (34) and then with Eric Davidson (33)) in mammalian DNA that were repeated many thousands of times. It was universally reasoned that genes encoding proteins would be present twice per diploid genome. All labeled RNA experiments attempting to show that hnRNA was a precursor to cytoplasmic mRNA by hybridization to DNA were defeated by the virtual impossibility of getting rid sufficiently of the repeated sequences, many of which are copied into RNA (33, 34).

However, progress in separating and characterizing the various nuclear RNAs was made by Sheldon Penman, who had come along with us to AECOM. With an assist from the cell biologist Eric Holtzman, they perfected a technique to separate completely the nucleolus containing the pre-rRNA from hnRNA (35). This allowed us to show clearly the very large size of much of the hnRNA and, when completely separated from pre-rRNA, showed that the base composition of all sizes of hnRNA reflected that of the majority of cellular DNA (36).

In addition, during these years, one positive step forward was made with regard to a possible relationship between large nuclear RNA and smaller polysomal mRNA. Sarah Stewart and Bernice Eddy at NIH discovered a small DNA virus termed poxyoma that could cause tumor formation in rodents (37). Subsequently, another similar small DNA virus, SV40, was also found to be capable of instigating tumor formation (37). Tom Benjamin, a graduate student in Renato Dulbecco’s group at the California Institute of Technology (Caltech), showed that the induced tumor cells contained polyoma-specific RNA (38). The Dulbecco group then demonstrated that SV40 DNA also was integrated into the DNA of transformed tumor cells (39).

Uno Lindberg, a Swedish postdoctoral fellow in our laboratory, followed up the Caltech experiments by demonstrating in SV40-transformed cells that the SV40-specific nuclear RNA was larger than SV40-specific polyribosomal RNA in either transformed or acutely infected cells. This was the first demonstration of a specific nuclear RNA, albeit virus-specific, that could represent a possible specific mRNA precursor (40).

Columbia University (1968–1974)

In 1967, one of the great friends I had made at MIT, Cyrus (Cy) Levinthal, called me to say, “We’re going to move to Columbia and reclaim Morgan’s department. Meet me at Yale, where we’ll see a model for a new building.” What he was talking about, of course, was the Biology Department that, in the 1930s, was led by Thomas Hunt Morgan and his famous students, Alfred Sturtevant, Hermann J. Muller, and Calvin Bridges. Their studies on Drosophila laid the genetic foundation for modern biology. (This history is beautifully described by Carlson (41).)

When Cy called, I was setting up a succrose gradient and, in my ignorance, did not get it for a bit. After joining him to see the new Kline Biology Tower at Yale University and hearing the promises made to him by David Truman, the Columbia provost, I decided to join him. For six months or so, another good friend of ours, Jerry Hurwitz, a star biochemist at AECOM (and Salva Luria’s brother-in-law), had also planned to come with us, but Jerry changed his mind.

The spring of 1968 saw nationwide campus upheavals against the Vietnam War, and although I sympathized with the protests, renovations of my new laboratory were delayed, slowing down the move to Columbia. Finally, after I visited a plumbing supply store, buying and delivering some PVC (polyvinyl chloride) pipes, the plumbing for our sterilizer was finally completed, and the laboratory was moved. Six very busy years followed.

The first big break came out of experiments I was doing myself with my technician Bob Tushinski. We had made both $^3$H]uridine- and $^3$H]adenosine-labeled polyribosomal mRNA that I was using in experiments that included digestion of the mRNA with ribonuclease A. The enzyme digested to cold acid (5–10% TCA) solubility >99% of the $^3$H]uridine-labeled mRNA, but a resistant fraction of ~5% of the $^3$H]adenosine-labeled mRNA was not digested. The percentage of the resistant RNA was not
constant but rose with decreasing size of the mRNA, suggesting perhaps the same size adenine-rich unit in all mRNAs.

I knew that pancreatic RNase did not digest (at least well, if at all) ApA stretches. Furthermore, I knew that Tom August, an associate of Hurwitz’s at AECOM, had been studying the fact that E. coli RNA polymerase given only ATP made poly(A) without a template (42). More important, Mary Edmonds and Rich Abrams had found a lymphocyte poly(A) polymerase also requiring no template (43), and George Brawerman had found a poly(A) segment in liver cells (44). However, neither laboratory had determined what type of cellular RNA might contain poly(A). In 1971, both the Edmonds and Brawerman laboratories isolated labeled polysomal mRNA and found the poly(A) segment, as we had also done. Finally, we all knew it was mRNA that contained the poly(A). Three papers published in the Proceedings of the National Academy of Sciences of the United States of America in June 1971 described all of our results (45–47). In the following year or so, it was established that the poly(A) was added within <1 min of labeling with [3H]adenosine to the 3′-end of hnRNA molecules (48–51), followed within 5–10 min by the appearance of exactly the same size 3′-poly(A) unit in polyribosomal mRNA in the cytoplasm. These results were the first strong indication of conversion of at least some part of cellular hnRNA to mRNA (50–52).

A second big redirection in our efforts also took place at Columbia. In addition to the small transforming viruses SV40 and polyoma, a number of laboratories demonstrated that adenovirus, a common human DNA virus that causes upper respiratory infections, could also cause cell transformation through incorporation of a small fragment of its DNA into host cells. This virus was observed in EM by Sam Dales (53) to “dump” its DNA into the cell nucleus, where the DNA replication occurred. It seemed likely that, even during the late lytic phase, the adenovirus DNA was copied by cellular RNA polymerases. 30–50% of the total pulse-labeled nuclear RNA was in fact adenovirus-specific, and many different virus-specific mRNAs were found in the cytoplasm. (Bob Roeder had described RNA polymerases I–III only three years before poly(A) was found in cellular mRNA. It was polymerase II that made hnRNA and would be the likely enzyme to copy adenovirus DNA (54).)

Lennart Philipson, an old friend whose laboratory in Uppsala, Sweden, was engaged in studying adenovirus structure and replication, took a working sabbatical in our laboratory beginning in February 1971. We decided to look into the details of adenovirus mRNA formation. We had just finished our initial work with cellular poly(A), and within a short while, we showed that adenovirus mRNA emerged from the nucleus of infected cells with exactly the same length 3′-poly(A) tail as that in cellular mRNA (55). Moreover, the total virus-specific nuclear RNA from infected cells was very large and could pre-occupy all of the sites in the DNA to which labeled smaller cytoplasmic poly(A)+ adenovirus mRNA could hybridize: so-called hybridization competition. Moreover, it was also clear the [3H]uridine-labeled nuclear adenovirus RNA was much larger than the cytoplasmic adenovirus mRNA and that only about one-half of the virus-specific nuclear RNA could be competed by the cytoplasmic adenovirus mRNA (56).

Based on the adenovirus results, the situation seemed ripe for exploitation of the hypothesis that a larger nuclear precursor is processed to smaller mRNA, discard ing a large fraction of the primary transcript. By this time (1973), restriction enzymes that allowed definition of ordered fragments of genomes had been discovered (particularly useful initially for studying DNA viruses). By restricting labeling times to very short exposures (1–2 min), we hoped to be able to label nascent nuclear RNA chains at their growing ends, separate them by sedimentation analysis into successively small to large sizes, and hybridize the sized RNA fractions to the ordered adenovirus DNA fragments, thus identifying the starting site (regions) for synthesis of the shorter virus-specific mRNA as well as the ultimate length of the primary transcript. Steve Bachenheimer, a postdoctoral fellow, had begun such experiments when we made the final intra-city move from Columbia University to Rockefeller University in 1974. (While I was teaching a course each semester at Columbia, I was not required to teach at Rockefeller. By choice, however (I guess the urge to be useful to students), my “free time” was soon devoted to writing textbooks, and after 1980, I took part for twenty-seven years in organizing and teaching a course at Rockefeller called “Gene Expression.”)

Rockefeller University Years (1974–Present)

Over the decades at Rockefeller University, our group has worked in three areas, all directly connected to my original 1961 aim: how do mammalian cells make and control the making of different mRNAs?

Primary Pre-mRNA Transcripts—Much of the initial progress in learning about pre-mRNA transcripts in animal cells came originally from studying adenovirus mRNA formation. By 1975, in work performed largely in Philipson’s laboratory in Sweden and by a large group at
Cold Spring Harbor (CSH), the adenovirus genome was dissected with restriction enzymes into ordered fragments to which different early and late mRNAs specifically hybridized (57, 58). A workable technique for strand separation of the ~36-kb genome and its pieces was achieved (59), and the transcribed strand(s) were identified. One striking result emerging from these efforts was that many of the numerous late (15–18 h after infection) adenovirus mRNAs were all copied from the rightward-reading strand (57, 58).

In our laboratory, using brief labeling periods (1 min), Bachenheimer pursued our strategy for mapping the site of origin and size of the late primary transcript, with the very suggestive result that a single very large (~30 kb) primary transcript existed late in infection (60). This large transcript contained sequences that included all of the late mRNAs plus spaces in between the identified mRNAs. Using isolated nuclei to elongate already initiated chains, Jeff Weber and Warren Jelinek confirmed and solidified the in vivo results of a single potential precursor to the late mRNAs. This primary transcript began ~5800 nucleotides from one end (the left) and extended almost to the other end (far right) of the genome (61).

It had been established in 1974 and 1975 with bacteriophage (62) and with mammalian pre-rRNA (63) that, after in vivo UV damage to a transcribing genome, the most distal portion of any new transcript was the first to be lost, followed in turn by sequences back to the promoter. Seth Goldberg, Weber, and Joe Nevins showed that the long adenovirus transcript was in fact the obligatory precursor to the late mRNAs by UV inactivation experiments (64, 65). Indeed, the most UV light-sensitive late adenovirus mRNAs mapped at the right end of the genome, and the other mRNAs showed successively less and less UV sensitivity moving toward the left end of the genome (66).

These experiments all together presented a strong case for processing the major late transcript to make the late adenovirus mRNAs (66). Furthermore, the labeling and UV experiments made the remarkable EM pictures taken in 1977 at CSH by Louise Chow and Tom Broker together with Rich Roberts (67) and at MIT by Susan Berger and Claire Moore together with Phil Sharp (68) immediately interpretable. Their pictures revealed that each late mRNA contained 3′-segments (leaders arising from sequences ~5800, 7200, and 9400 nucleotides from the left end of the genome) attached to a downstream “body” (coding region), all terminated by poly(A). As was soon discovered, splicing (as the “cut and paste” process was called) plus 3′-poly(A) addition illustrated how almost all eukaryotic mRNAs are formed from a longer primary RNA transcript.

Cell-specific mRNA Formation after Splicing—With the events of mRNA formation finally outlined after fifteen years of intense effort of many different scientists, my group and I turned to the obvious task that I had thought I might be working on in the 1960s: the basis of cell-specific mRNA formation and the proof or disproof (which never seemed likely) that transcriptional control was behind it all. Eva Derman spearheaded the initial effort by making a library of liver cDNAs (by the late 1970s, a commonplace event), a set of which were present in the liver and not other tissues (69). Using this library, Don Powell and Jeff Friedman showed very clearly that transcription in the nucleus of the genes encoding the liver-specific mRNAs was also specific to the liver (70). At least the primary basis of cell-specific control was transcriptional.

As did many other laboratories, we used the developing knowledge that sequences upstream of the start site of transcription contained sites responsible (at least partly) for correct transcription initiation to detect proteins that bound the presumably transcriptional regulatory sequences. Choosing such binding proteins that were enriched in liver nuclear extracts (not necessarily “specific” to liver cells), we purified several of these proteins and cloned their genes (71–73). Like those in many other laboratories, these experiments, using many different tissues, provided a useful entry point for the study of dozens of transcriptional regulatory proteins of the so-called “activator” type, i.e. required for transcription initiation.

However, we were put on a parallel but different path by experiments conducted mainly by David Clayton, a graduate student. There is a long history of study of disaggregated parenchymal liver cells obtained by perfusion through the portal vein of a disaggregating solution (e.g. containing the Mg2+ chelator EDTA), removal of the liver capsule, and recovery of released hepatocytes. David did this and scored transcription of the liver-specific mRNAs (by the chain elongation method in isolated nuclei). Although the initially disaggregated cells still transcribed all pre-mRNAs for 1–2 h in culture, by 24 h in culture, they had lost >50–90% of transcription of most liver-specific transcripts. The cells were still viable from the point of view of making liver-specific proteins from pre-existing mRNAs (74).

Clayton took an additional step. After perfusion, which would have released individual hepatocytes, he switched
to a perfusing medium that allowed cell reattachment. When thin slices of such livers (as well as slices of normal liver perfused only with cell culture medium) were placed in culture, the nuclei still synthesized liver-specific mRNAs like untreated cells after 24 h (75). It appeared that cell-specific attachments dictated transcriptional specificity. We did not know what cell attachments might underlie transcriptional specificity in the liver, and of course, different genes might require different attachments. We went looking for a simpler situation in which a specific cell surface receptor and an identified ligand, available in pure form, might allow an inroad into this problem.

**Signaling Transcriptional Regulation from the Cell Surface: The JAK-STAT Pathway**—The first extracellular gene signaling that changed the rate of transcription of a specific gene in mammalian cells was shown with estrogen stimulation of the integrated DNA of mouse mammary tumor virus (76, 77). However, this did not solve our problem because estrogen was known to diffuse into cells. By 1980, dozens of cell surface receptors were identified for circulating proteins (cytokines and growth factors), and it was hypothesized that these extracellular ligands triggered changes in gene expression. However, the basis for the changing mRNA levels after exposure to surface ligands was, to say the least, unsettled.

A former postdoctoral fellow and good friend, Ernest (Pete) Knight had purified β-interferon (β-IFN), which was known to induce virus resistance without entering cells in an RNA synthesis-dependent manner (78). We teamed up with Pete and established that the specific pre-mRNAs of IFN-induced mRNAs indeed showed greatly enhanced transcription within 15–30 min of IFN treatment (79). Moreover, the proteins required for increasing transcription were already present in the cells; the IFN outside the cell somehow activated the pre-existing protein(s) inside the cell (80). As we had done with liver-specific promoters and cognate transcription factors, we identified binding sites on activated genes and the proteins in IFN-treated cells that bound such sites (81, 82), purified the proteins, and cloned their genes (83–85). The IFN-responsive transcription factors were discovered to be phosphorylated on a single tyrosine after IFN treatment (86, 87). Because these were dual-purpose proteins, *i.e.* signal transducers and activators of transcription, they were named STATs (reviewed in Ref. 88).

At the same time as our experiments with IFN were proceeding, George Stark, already a great friend and ultimately a collaborator, was engaged in a search for mutants that rendered cells nonresponsive to IFN. He and colleagues cloned the DNA of a series of the relevant mutated genes (89–91). We sent George the cDNAs for STAT1 and STAT2, and two of the genes they had found mutated to IFN nonresponsiveness encoded STAT1 and STAT2 (92, 93). Of equal importance, they had cloned two other genes that turned out to encode tyrosine kinases of the type earlier identified by Andy Wilks (who named them JAKs) and John Krolewski (12, 94, 95). Thus, together (reviewed in Ref. 88), we had learned about cell surface activation by a cytokine and the internal proteins in the cell that conveyed that activation message to the nucleus. A family of seven STATs and a family of four JAKs that function in response to many different cytokines and growth factors were soon identified. The many cogent experiments supporting these and later conclusions about cytokines have recently been discussed at length on the occasion of the twentieth anniversary of the recognition of the JAK-STAT pathway (reviewed in Ref. 88).

**Conclusion**

At the risk of undermining this whole effort with unsolicited advice, I wish to close with some personal and not terribly original thoughts that just might be of some help to younger scientists. The monumental advances in biological, genetic, biochemical, and structural science of the past sixty years have often been enumerated. These advances occurred with the dedicated and tireless efforts of individual “bench scientists” (among which, for thirty of the fifty-plus years I have had a laboratory, I count myself) plus very occasional inventive and visionary leaders. During this period, all of us were gifted with constantly improving technology, including, most recently, the means of collecting and handling huge amounts of information.

It is possible now to reasonably suggest a very large fraction (never “all” it seems) of the parts list, and at least a good many of the wiring diagrams of cells are in hand. Never again will the general structure of DNA be initially discovered or the *first* whole genome sequence be accomplished or the repeated use of helices and β-sheets to construct proteins be first recognized. Maybe even most of the types of RNAs have been recognized. However, the young need not despair. Take the attitude that your predecessors have cleared the decks of a huge list of chores. How all these items fit together to operate cells under changing and demanding circumstances, “systems biology,” a term favored by some, is hardly finished. How concerted cellular communication and cooperation produce cell specificity, construct organisms, and guarantee survival, repro-
duction, and the ultimate cellular specialization, i.e. brain function and consciousness, all await solution, and you will get there, step by step.

Finally, I offer one hint from Benjamin Franklin that, in my opinion, should inform the behavior of all scientists: “A man (woman) wrapped up in himself (herself) makes a very small bundle” (my italics). We are all needed and are all in this together.

Acknowledgments—In putting this piece together, I have acknowledged by name approximately only thirty or so colleagues. I hope that the 100 or so I have not specifically mentioned realize I am deeply admiring of and indebted to every one of them also.

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REFERENCES

1. Johnson, J. C., and Stollerman, G. H. (1969) Nephritogenic streptococci. Annu. Rev. Med. 20, 315–322
2. Morse, S. I., Darnell, J. E., Jr., and Glaser, R. J. (1954) Production of streptococcal infections in pharynx of rabbit. Proc. Soc. Exp. Biol. Med. 87, 582–585
3. Glaser, R. J., Thomas, W. A., Morse, S. I., and Darnell, J. E., Jr. (1956) The incidence and pathogenesis of myocardin in rabbits after group A streptococcal pharyngeal infections. J. Exp. Med. 103, 173–188
4. Darnell, J. E., Jr., Pesch, B. B., and Glaser, R. J. (1955) Effect of penicillin on group A streptococci in vivo in the absence of leucocytes. J. Clin. Invest. 34, 1237–1241
5. Eagle, H. (1955) Nutrition needs of mammalian cells in tissue culture. Science 122, 501–514
6. Eagle, H., Piez, K. A., Fleischman, R., and Oyama, V. I. (1959) Protein turnover and the ultimate cellular specialization, i.e. brain function and consciousness, all await solution, and you will get there, step by step.

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50. Darnell, J. E., Philadelphia, L., Wall, R., and Adesnik, M. (1971) Polyadenylic acid sequences: role in conversion of nuclear RNA into messenger RNA. Science 174, 970–977.

51. Nakazato, H., Kopp, D. W., and Edmonds, M. (1973) Localization of the polyadenylate sequences in messenger ribonucleic acid and in the heterogeneous nuclear ribonucleic acid of HeLa cells. J. Biol. Chem. 248, 1472–1476.

52. Jelinek, W., Adesnik, M., Salldit, M., Sheiness, D., Wall, R., Molloy, G., Philadelphia, L., and Darnell, J. E. (1973) Further evidence on the nuclear origin and transfer to the cytoplasm of polyadenylic acid sequences in mammalian cell RNA. J. Mol. Biol. 75, 515–532.

53. Dales, S. (1973) Early events in cell-animal virus interactions. Bacteriol. Rev. 37, 103–135.

54. Roeder, R. G., and Rutter, W. J. (1969) Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. Nature 224, 234–237.

55. Philadelphia, L., Wall, R., Glickman, G., and Darnell, J. E. (1971) Addition of polyadenylic acid sequences to virus-specific RNA during adenovirus replication. Proc. Natl. Acad. Sci. U.S.A. 68, 2806–2809.

56. Wall, R., Philadelphia, L., and Darnell, J. E. (1972) Processing of adenovirus specific nuclear RNA during virus replication. Virology 50, 27–34.

57. Philadelphia, L., Pertessson, U., Lindberg, U., Tibbets, C., Vennstrom, B., and Persson, T. (1975) RNA synthesis and processing in adenovirus-infected cells. Cold Spring Harb. Symp. Quant. Biol. 39, 447–456.

58. Sharp, P. A., Gallimore, P. H., and Flint, S. J. (1973) Mapping of adenovirus 2 RNA sequences in lytically infected cells and transformed cell lines. Cold Spring Harb. Symp. Quant. Biol. 39, 457–474.

59. Tibbets, C., and Pertesserus, U. (1974) Complementary strand-specific sequences from unique fragments of adenovirus type 2 DNA for hybridization-mapping experiments. J. Mol. Biol. 88, 767–784.

60. Batchenheimer, S., and Darnell, J. E. (1975) Adenovirus-2 mRNA is transcribed as part of a high-molecular-weight precursor RNA. Proc. Natl. Acad. Sci. U.S.A. 72, 4445–4449.

61. Weber, J., Jelinek, W., and Darnell, J. E. (1977) The definition of a large viral transcription unit late in Ad2 infection of HeLa cells: mapping of nascent RNA molecules labeled in isolated nuclei. Cell 10, 611–616.

62. Hackett, P. B., and Sauerbier, W. (1974) Radiological mapping of the ribosomal RNA transcription unit in E. coli. Nature 231, 639–641.

63. Hackett, P. B., and Sauerbier, W. (1975) The transcriptional organization of the ribosomal RNA genes in mouse I cells. J. Mol. Biol. 91, 235–256.

64. Goldberg, S., Weber, J., and Darnell, J. E. (1977) The definition of a large viral transcription unit late in Ad2 infection of HeLa cells: mapping by effects of ultraviolet irradiation. Cell 10, 617–621.

65. Goldberg, S., Nevins, J., and Darnell, J. E. (1978) Evidence from UV transcription mapping that late adenovirus type 2 mRNA is derived from a large precursor molecule. J. Virol. 25, 806–810.

66. Darnell, J. E., Evans, R., Fraser, N., Goldberg, S., Nevins, J., and Darnell, J. E. (1978) Interferon-stimulated transcription: isolation of an inducible gene and identification of its regulatory region. Proc. Natl. Acad. Sci. U.S.A. 83, 8925–8933.

67. Reich, N., Evans, R., Levy, D., Fahey, D., Knight, E., Jr., and Darnell, J. E. (1987) Interferon-induced transcription of a gene encoding a 15-kDa protein depends on an upstream enhancer element. Proc. Natl. Acad. Sci. U.S.A. 84, 6394–6398.

68. Fu, X.-Y., Kessler, D. S., Veals, S. A., Levy, D. E., and Darnell, J. E. (1990) ISGF3, the transcriptional activator induced by interferon α, consists of multiple interacting polypeptide chains. Proc. Natl. Acad. Sci. U.S.A. 87, 8555–8559.

69. Schindler, C., Fu, X.-Y., Improta, T., Abeersold, R., and Darnell, J. E. (1992) The proteins of ISGF-3, the interferon-α-induced transcriptional activator, define a gene family involved in signal transduction. Proc. Natl. Acad. Sci. U.S.A. 89, 7840–7843.

70. Schindler, C., Fu, X.-Y., Improta, T., Abeersold, R., and Darnell, J. E. (1992) Proteins of transcription factor ISGF-3: one gene encodes the 91- and 84-kDa ISGF-3 proteins that are activated by interferon α. Proc. Natl. Acad. Sci. U.S.A. 89, 7836–7839.

71. Schindler, C., Shuai, K., Prezzioso, V. R., and Darnell, J. E. (1992) Interferon-dependent tyrosine phosphorylation of a latent cytoplastic transcription factor. Science 257, 809–813.

72. Shuai, K., Stark, G. R., Kerr, I. M., and Darnell, J. E. (1993) A single phosphotyrosine residue of Stat91 required for gene activation by interferon-γ. Science 264, 1744–1746.

73. Stark, G. R., and Darnell, J. E. (2012) The JAK-STAT pathway at twenty. Immunity 36, 503–514.

74. Pellegrini, S., John, I., Shearer, M., Kerr, I. M., and Stark, G. R. (1989) Use of a selectable marker regulated by an interferon to obtain mutations in the signaling pathway. Mol. Cell. Biol. 9, 4605–4612.

75. Velazquez, L., Fellous, M., Stark, G. R., and Pellegrini, S. (1992) A protein tyrosine kinase in the interferon-α/β signaling pathway. Cell 70, 313–322.

76. Müller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, A., Silvernoven, O., Harpur, A. G., Barbiener, G., Witthuhn, B. A., Schindler, C., Pellegrini, S., Wilks, A. F., Ilh, N., Stark, G. R., and Kerr, I. M. (1993) The protein tyrosine kinase JAK1 complements defects in interferon-α/β and γ signal transduction. Nature 366, 129–135.

77. Müller, M., Laxton, C., Briscoe, J., Schindler, C., Improta, T., Darnell, J. E., Jr., Stark, G. R., and Kerr, I. M. (1993) Complementation of a mutant cell line: central role of the 91 kDa polypeptide of ISGF3 in the interferon-α and γ signal transduction pathways. EMBO J. 12, 4221–4228.

78. Leung, S., Qureshi, S. A., Kerr, I. M., Darnell, J. E., Jr., and Stark, G. R. (1995) Role of STAT2 in the α interferon signaling pathway. Mol. Cell. Biol. 15, 1312–1317.

79. Wilks, A. F. (1989) Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. Proc. Natl. Acad. Sci. U.S.A. 86, 1603–1607.

80. Wilks, A. F., Harpur, A. G., Kurban, R. R., Ralph, S. J., Züchter, G., and Ziemiecki, A. (1991) Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. Mol. Cell. Biol. 11, 2057–2065.