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Essay

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Eukaryotic RNAs: Once More from the Beginning . . .

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I am profoundly grateful to the American Society for Cell Biology for the award to me and my colleague Sheldon Penman of the E.B. Wilson Medal. This occasion presents the opportunity for reflections on a 40-year search for indications of how RNA functions as an informational molecule in eukaryotic cells. As will be recounted, this quest began in the late 1950s, before the recognition of mRNA, by studying the biosynthesis of poliovirus, an RNA virus. The search continued through the molecular and biochemical definition of classes of nuclear and cytoplasmic RNA in both growing and virus-infected cultured mammalian cells and, subsequently, through studies on the basis of cell-specific mRNA expression in the liver. Finally, in the recent past our laboratory has concentrated on our original goal, the mechanism of recognition of extracellular signals with consequent gene regulation in the cell nucleus.

THE HARRY EAGLE LAB FROM 1956 THROUGH 1960

Fresh from a medical internship at Barnes Hospital in St. Louis, I arrived in July 1956 in the Section on Experimental Therapeutics, National Institute of Allergy and Infectious Diseases, headed by Harry Eagle. Previous work in Eagle's laboratory had explored the action of penicillin (Eagle, 1955a), a topic I had worked on in medical school (Darnell *et al.*, 1955). But by 1955 all of Eagle's efforts had become devoted to the monumental series of studies that defined simple, reproducible conditions for the successful continuous culture of human and mouse cell lines (Eagle, 1955b, 1959). Together with Ted Puck's development of facile clonal isolation techniques for cultured mammalian cells (Puck and Fisher, 1956; Puck *et al.*, 1956) and Renato Dulbecco's introduction of quantitative animal virology (Dulbecco and Vogt, 1954a,b), the way was open to begin the study of animal virus biochemistry. And this, Dr. Eagle emphatically declared, was what I should think about studying. Alas, I knew next to nothing about animal virology, aside from the rudimentary knowledge acquired about viral infections in medical school. Fortune provides for the lucky, and pressure of the military draft in the aftermath of the Korean War had delivered Robert I. DeMars to the Eagle lab just 6 months ahead of my arrival, and I was assigned a bench in the same room with him. DeMars, S.E. Luria's third graduate student, gave me his copy of Luria's *General Virology* (Luria, 1953), first edition, and with consummate patience taught me what I needed to know. After a year or so under Bob's watchful eye, I had settled in to study the rapidly cytolytic poliovirus, developed the first plaque assay on continuously growing mammalian cells, performed a one-step growth curve, proved all cells in the culture could be infected by plating cells as infectious centers, and determined the release pattern of the virus (Darnell, 1958; Darnell and Sawyer, 1959, 1960). That is, I followed in lockstep the experiments that a good phage worker prescribed.

Leon Levintow, an M.D. turned biochemist and another Eagle recruit to animal cell work, was studying the enzymology of asparagine formation (Levintow, 1957) and was looking for other challenges. Leon and I developed a purification scheme for poliovirus (Levintow and Darnell, 1960) using the newly minted cesium chloride density equilibrium banding technique developed by Jerry Vinograd (Meselson *et al.*, 1957), so that multiple parallel purifications of experimental virus samples was possible. We wished to study the formation of both the protein and the RNA of polio virus. Through Eagle's and Karl Piez's efforts, the ready exchangeability of the soluble pool of intracellular amino acids with amino acids in the medium had been demonstrated (Piez and Eagle, 1958). Knowing this, we could chart the time course of poliovirus protein biosynthesis by adding label at later and later times after infection, purifying the virus, and by the declining radioactivity in the later time samples estimate the time of formation of the protein component of the virus (Darnell and Levintow, 1960). However, it was not clear how to study nucleic acid accumulation biochemically. Infectious RNA from viruses and infected cells had been discovered, but only one in a million molecules could be shown to be infectious. The intracellular pools that fed RNA biosynthesis were unknown. I showed, however, that labeling with

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the nucleoside adenosine resulted in equal labeling of AMP, ADP, and ATP, and the three nucleotides remained equally labeled regardless of how long the label was applied. Thus regardless of what fed RNA synthesis, adenosine labeling of cells seemed an appropriate way to study RNA biosynthesis. Most important, the acid-soluble pool was essentially completely labeled within 20 min. Finally I found that when labeled adenosine was added at ~1 h, the specific activity of pool adenine and the specific activity of adenine in virus RNA purified from a 6-h sample were equal. Thus the synthesis of a specific RNA, i.e., polio RNA, could be charted by label incorporation. We measured the time course of poliovirus RNA synthesis using the same protocol as for the protein: infecting, labeling samples at intervals up to 8 h (maximal virus yield was achieved by that time), purifying samples, and plotting the decreasing concentration of radioactivity to give us the time course of RNA biosynthesis (Darnell *et al.*, 1960). Although these results were hardly earth shaking, they raised an enticing problem: the RNA growth curve preceded the protein growth curve by ~30 min, stimulating the wish to understand more precisely how viral protein synthesis possibly depended on prior RNA virus synthesis.

A SOJOURN IN PARIS

At this point a second training opportunity had opened and my wife Jane, three boys—5, 2, and ¼ years old—and I left for a single postdoctoral year in Paris under the tutelage of Francois Jacob. Before I left I was offered my first academic position, an assistant professorship at MIT by S.E. Luria, upon my return from Paris. Upon arriving in Paris in July 1961, I found everyone at the Pasteur Institute completely convinced by the "mRNA hypothesis." This new idea depended on the classic experiments of Brenner *et al.* (1961) and the foregoing genetics of regulated gene induction (Kaiser and Jacob, 1951) as put forward in the classic Jacob and Monod (1961) paper in the *Journal of Molecular Biology*. These experiments showed that all ribosomes were reprogrammed during T4 infection of *Escherichia coli* and that virulent mutants in bacteriophage λ as well as mutants in β-galactosidase induction in *E. coli* implied an intermediate between genes (DNA) and specific protein synthesis.

I'm afraid my goal in joining Jacob, namely, to learn genetics, was never completely accomplished. In fact I spent considerable time in teaching others to run sucrose gradients to fractionate RNA samples during that year. But my appetite for returning to MIT to work on the cellular biochemistry of animal cell mRNA was acute by the year's end. Besides, there was at the time no prospect of doing genetics on cultured mammalian cells anyway, and my years in Bethesda had convinced me I would always concentrate on biochemical approaches to animal cells.

ESTABLISHING A CELL CULTURE LAB AT MIT: pre-rRNA, hnRNA, AND POLYRIBOSOMAL mRNA

In the three short years I spent at MIT (June 1961–June 1964), I was joined by my first group of postdoctoral fellows, some my own age, and students only a few years younger, and together we had what I surely remember as an exhilarating time. For this narrative we can only recount some of the highlights. Klaus Scherrer, the first postdoc to join me, and I developed a "hot phenol" (60°C) technique for extracting cell RNA in which close to 100% of the RNA was extracted (Scherrer and Darnell, 1962; Scherrer *et al.*, 1963). This was the case whether the RNA was labeled overnight and was mostly cytoplasmic or labeled for 5 min, when it was almost exclusively nuclear. When the labeled, extracted RNA was separated on sucrose gradients, the long-labeled RNA matched the OD_{260} profile, and the three peaks of RNA recognized by this time were 18 and 28S rRNA and the 4S peak of tRNA. When we sedimented briefly labeled RNA (5, 15, and 30 min, for example), a somewhat startling discovery was made: the newly labeled (nuclear) RNA did not match the preexisting RNA in sedimentation profile, and most of the newly labeled RNA sedimented faster than rRNA (Scherrer and Darnell, 1962; Scherrer *et al.*, 1963). Moreover, when the label time was longer than ~10 min, discrete peaks appeared in the profile, first at 45S and later at 32S. When actinomycin D was used to stop further synthesis of RNA after 5 min, the largest peaks of RNA disappeared, and a large fraction of the radioactive RNA reappeared at 28 and 18S (Girard *et al.*, 1964). No chemical characterization of the RNA other than the average base composition was available to us at the time, so we compared the newly labeled large 45S peak and the preexisting rRNA (Darnell *et al.*, 1963). The average rRNA composition was 60% G+C (the 28S was slightly higher in G+C than the 18S) and the large 45 and 32S peaks had a similar high G+C content like the rRNA. We concluded from the actinomycin chase plus the base composition that the 45 and 32S peaks were pre-rRNA molecules and proposed that *RNA processing* was required to reduce the *primary transcript* to usable form in the cytoplasm where finished ribosomes functioned.

We now jump ahead chronologically to achieve some scientific unity in this discussion. To complete this early chapter of our studies on ribosome formation, we describe work that was done mainly by Sheldon Penman (Penman, 1966) and Jon Warner (Warner, 1966) after all of our group had moved to the Albert Einstein College of Medicine in New York. Sheldon became interested in localizing the site of pre-rRNA in

cells. From much earlier work the nucleolus of the cell was expected to be the site of ribosome formation. Sheldon worked out a separation technique using a high-monovalent salt concentration to disperse chromatin, followed by DNase treatment to break the resulting DNA gel. This resulted in fragile nuclei, bounded by what later became recognized as lamins (Gerace and Blobel, 1980) but retaining nucleoli and extranucleolar ribonucleoprotein (Holtzman *et al.*, 1966; Penman, 1966). The nuclei could be broken further by shear (vigorous pipetting) and nucleoli separated from the "nuclear sap." The nucleolar fractions clearly contained the 45 and 32S and small amounts of 18S rRNA (Penman, 1966). Jon Warner showed that nucleolar particles existed with ribosomal proteins and pre-rRNA in the nucleolus (Warner, 1966). We had earlier found (Girard *et al.*, 1965) that delivery to the cytoplasm of 18S rRNA and small ribosomal subunits was more rapid than the appearance of 28S rRNA, which meant that the maturation time of the small subunit was faster than the maturation time for the large subunit. Ernest "Pete" Knight and Jacques Pene also found that the 5S RNA that is part of a ribosome was not part of the primary transcript for rRNA (Knight and Darnell, 1967), but another molecule, the 5.8S molecule (originally called 7S), was a cleavage product in the maturation of the 28S RNA (Pene *et al.*, 1968). So it was clearly established that the nucleolus was a physical site for pre-rRNA synthesis, pre-rRNA processing, and ribosome assembly; the two subunits that arose from each primary 45S RNA transcript arrived in the cytoplasm separately.

Putting aside ribosomal precursor RNA and ribosome formation, we now revert back to the RNA studies at MIT that were aimed at finding the origin of mRNA. Once the idea of mRNA was coined in Paris, it was only a question of time before this RNA species could be identified and studied in animal cells—or so I thought upon returning to MIT in June 1961. When the largest or most briefly labeled RNA in the nucleus was analyzed for base composition, it had a G+C content of ~42%, close to the base distribution in the total mammalian cell DNA and very unlike that in the rRNA. Thus, the key question from the initial discovery of the large *heterogenous nuclear RNA*, the hnRNA fraction as we called it, was its relationship to mRNA. The first step in solving this problem was clearly to identify physically the mRNA fraction. This was accomplished through the association of mRNA with the demonstrable site of amino acid incorporation, the polyribosomes. Jon Warner and Paul Knopf at MIT (students of Alex Rich's), Hans Noll in Pittsburgh, and Alfred Gierer in Germany all had independently discovered polyribosomes (Gierer, 1963; Warner *et al.*, 1963b; Wettstein *et al.*, 1963). Warner and Knopf worked on the seventh floor of the building, where our group worked on the eighth floor at MIT, and Warner and I had tried to put ribosomes onto poliovirus RNA before he found polyribosomes in reticulocyte extracts (Warner *et al.*, 1963a). So, we knew very early to search in polyribosomes for mRNA. Sheldon Penman, Klaus Scherrer, and I decided to make extracts of HeLa cells to search for polyribosomes and soon found them (Penman *et al.*, 1963). Moreover, in cells infected by poliovirus, the cell polysomes (with 3–12 ribosomes) disappeared, and large virus polysomes (with >25 ribosomes) took their place (Rich *et al.*, 1963). This indicated that polyribosomes assembled on whatever mRNA was being translated in the cytoplasm. We were encouraged to label cells briefly so that no mature rRNA would be labeled in an attempt to isolate newly labeled mRNA from polyribosomes (Penman *et al.*, 1963). In this experiment we identified RNA that ranged in size from a few hundred to 5000 bases in length with a mean of ~1500 bases. Although this presumptive mRNA was much shorter than hnRNA, it had a base composition very similar to hnRNA. The problem was clearly defined. Was the hnRNA the nuclear precursor to mRNA just as pre-rRNA was the precursor to rRNA?

THE ALBERT EINSTEIN COLLEGE OF MEDICINE AND COLUMBIA YEARS: POLY(A) IN hnRNA AND THEN mRNA

Further experiments at Albert Einstein College of Medicine in the late 1960s showed the DNA-like hnRNA existed outside the nucleolus (Soeiro *et al.*, 1966; Warner *et al.*, 1966), and we began grappling with ideas that would relate specific nucleotide sequences in hnRNA to sequences subsequently found in mRNA. Uno Lindberg, a Swedish postdoctoral fellow, showed that in SV40-infected cells, the average size of SV40-specific nuclear RNA (detected by hybridization to SV40 DNA) was larger than the SV40-specific mRNA in polyribosomes (Lindberg and Darnell, 1970). Thus processing of large to small RNAs during mRNA formation was certainly possible.

We then began a lengthy series of hybridization and hybridization competition experiments with total hnRNA and total mRNA designed to test their sequence relatedness (Soeiro and Darnell, 1969, 1970). Sad to say, these quite difficult experiments proved inconclusive in relating hnRNA to mRNA, but they did show clearly one point: there were "repetitive" or "rapidly hybridizing" RNA sequences in hnRNA that hybridized to sequences scattered in all chromosomes (Pagoulatos and Darnell, 1970). These sequences were much more plentiful in hnRNA than in mRNA, suggesting their removal in reducing the hnRNA to the protein coding mRNA (Darnell and Balint, 1970).

During these experiments we moved the lab to Columbia University, which in the context of this discussion prompted by the E.B. Wilson award, deserves a

momentary digression. Our new lab was to be situated in renovated space on the sixth floor of an extension to Schermerhorn Hall that had been erected about the turn of the century (the 19th to 20th century, that is). It was rumored at the time, but unverified, that E.B. Wilson himself may have occupied this space. Being 37 years old at the time, and admittedly not historically oriented, I saw old cabinets removed, old slides behind them discarded, and thought about little more than the tardiness of the construction crew in getting the new lab installed. In early December 1998, knowing I was soon to visit San Francisco to give this lecture and having reread a fair amount about Prof. Wilson, I decided to check on the 1968 rumors. The Dean of the Graduate School, Eduardo Macagno, was kind enough to search files and found a 1919 directory of alloted space. Sure enough, Prof. Wilson had his office in Schermerhorn extension, room 608, and his labs nearby. We had in fact unknowingly occupied E.B. Wilson's rooms. Perhaps this location brought us good luck, because we shortly made a discovery that was the first substantial step in unlocking the hnRNA-to-mRNA puzzle.

In the course of the RNA–DNA hybridization experiments begun at Albert Einstein College of Medicine and continued after we moved the lab to Columbia, we had prepared both tritiated adenosine-labeled polyribosomal mRNA and tritiated uridine-labeled mRNA. After the RNA–DNA hybridization reactions were concluded, we treated them with pancreatic ribonuclease to destroy the unpaired mRNA. As a control we digested the labeled mRNA samples without any DNA present. The adenosine-labeled mRNA had an RNase-resistant core that was not present in the uridine-labeled samples. This material was, of course, the poly(A) tail of the mRNA (Darnell *et al.*, 1971b). Moreover, the hnRNA fractions also contained a proportionally smaller amount of adenosine-labeled resistant material. When we examined by gel electrophoresis the size of this adenosine-labeled RNA after brief label times (<30 min), the size of the nuclear and cytoplasmic resistant fractions was identical, ~200–250 nucleotides (Darnell *et al.*, 1971a; Edmonds *et al.*, 1971). Finally, very brief label times (1–2 min) showed this material was labeled as part of hnRNA before it appeared in the cytoplasm, indicating a direct relationship between the two (Jelinek *et al.*, 1972). The poly(A) was shown to be at the 3′ end of the mRNA, the most convincing experiment being the demonstration of one 3′ adenosine per ~200 AMPs in alkali hydrolyzates of purified poly(A) (Molloy and Darnell, 1973). This discovery of poly(A) in our lab was paralleled exactly in time by work in Mary Edmonds's lab in Pittsburgh (Edmonds *et al.*, 1971) and George Brawerman's lab in Boston (Lee *et al.*, 1971). Each of those groups also had made earlier findings relating to poly(A). Edmonds found an enzyme in rat liver that synthesized poly(A) adenylic acid (Edmonds and Abrams, 1960; Edmonds and Caramela, 1969), and Brawerman had demonstrated poly(A) in cytoplasmic RNA of rat liver (Hadjivassiliou and Brawerman, 1967). Joe Kates had also made similar findings both with vaccinia virus mRNA and cell RNA, but his 1970 Cold Spring Harbor report was not published until early 1971 (Kates, 1970). But the realization that a unit-sized poly(A) was in fact part of the polysomal mRNA fraction of mammalian cells and also was present first in hnRNA was simultaneously arrived at in the Edmonds lab and in our lab in Columbia. At this point we felt secure that an important RNA-processing step had been uncovered that occurred in the formation of the 3′ ends of most mammalian RNAs. [As usual in nature, there are no universals; Milton Adesnik soon found that mammalian histone mRNAs do not contain a poly(A) (Adesnik and Darnell, 1972).]

ROCKEFELLER UNIVERSITY (1974 TO ?): CAPS AND POLY(A) IN LARGE hnRNA AND THE ADENOVIRUS MAJOR LATE TRANSCRIPT

The next major discovery of the distinguishing chemical features in mammalian mRNA was made approximately at the same time in several virology laboratories (Furuichi and Miura, 1975; Furuichi *et al.*, 1975a; Wei and Moss, 1975). A number of animal viruses contain the enzymatic machinery for synthesizing their own mRNAs within the particle itself. The mRNA synthesized by these virions was shown to begin with a blocked, methylated nucleotide structure, m^7GpppNp. In discussions with Aaron Shatkin in the summer of 1994, he explained these new findings to me and suggested that we should examine HeLa cell RNA for caps, as the 5′ end structure came to be called, especially since Robert Perry (Perry and Kelley, 1974) had described methyl groups in the mRNA of mouse cells earlier that year. We therefore labeled HeLa cells with [^{35}S]methionine, the precursor to *S*-adenosyl methionine that contributes methyl groups to RNA, and in short order found caps in RNase digests of HeLa cell polysomal mRNA (Furuichi *et al.*, 1975b). We showed that poly(A)-containing hnRNA also contained caps (Salditt-Georgieff *et al.*, 1976). At the end of these experiments (published in February 1976) we had clear knowledge that poly(A)-containing mRNA had one cap and one poly(A) per 1500 bases, whereas the polyadenylated fraction of hnRNA had an average of one cap and one poly(A) per 5000 bases. And in fact the largest hnRNA had one cap and one poly(A) per 10,000 bases.

During this time (1975–1976) we had begun an effort to sort out the details of mRNA processing by studying adenovirus mRNA formation in the cell nucleus (Bachenheimer and Darnell, 1975). We knew that adenovirus DNA entered the cell nucleus and that the

resulting mRNA was both capped and polyadenylated (Philipson *et al.*, 1971; Sommer *et al.*, 1976). We used the then newly developed techniques of restriction enzyme digestion to break the adenovirus DNA into pieces and prepared very briefly labeled nuclear RNA from isolated nuclei. The growing RNA chains, labeled only in their 300–500 terminal nucleotides, were separated according to size and hybridized to an ordered array of adenovirus DNA fragments (Bachenheimer and Darnell, 1975; Weber *et al.*, 1977). The shortest RNA hybridized to a region ~5500 nucleotides from one end of the linear adenovirus genome, with longer and longer RNA hybridizing to fragments all the way to the far end of the 36-kb genome. We also used UV irradiation, which blocks promoter distal compared with promoter proximal RNA synthesis. The same result was obtained; i.e., synthesis originated near ~5500 nucleotides from one end (Goldberg *et al.*, 1977). Thus we had mapped the primary transcript from adenovirus DNA as a giant ~30-kb molecule. It was the electron microscopic study of adenovirus mRNAs hybridizing to the region encoding this major late transcript that uncovered splicing. The labs of Philip Sharp (Berget *et al.*, 1977) and a group of workers at Cold Spring Harbor (Chow *et al.*, 1977) both found evidence that the 5′ ends of the late adenovirus mRNAs contained short regions from the 5500-nucleotide site and also contained sequences far downstream all the way to the 30,000-bp, distant right end of the genome. In view of the existence of the long adenovirus primary transcript, these two groups proposed a splicing reaction that brought the mRNA sections together.

Despite biochemical evidence from HeLa cell nuclear RNA (>10 kb capped and polyadenylated molecules) and knowing of the existence of adenovirus major transcript, it had remained too great a leap of imagination for our group to suggest that the two mRNA signposts, the 5′ cap and the 3′ poly(A), were brought together in the smaller mRNA by splicing, leaving out intervening sequences. Rather, the discussion in our papers concentrated on how mRNA might be processed from each end of the large capped, poly(A)-containing nuclear molecule. We were still in the grips of an earlier era of molecular biology in which an important watchword was *colinearity*, the central tenet of which was that an mRNA transcript would match the stretch of DNA from which it was copied.

mRNA REGULATION IN THE LIVER AND IN RESPONSE TO CYTOKINES

With full knowledge of how mRNA was made, our thoughts could finally be focused on the topic that had motivated our work since the training period in Paris with Francois Jacob. How was gene regulation in animal cells executed? It was clearly expected that transcriptional control would be important. For example, mRNAs, such as globin mRNA, seemed to be present only in red blood cells. But the extent to which post-transcriptional regulation in the nucleus or regulation of mRNA stability in the cytoplasm might contribute to changing mRNA levels was unclear. We undertook testing the extent of transcriptional control by studying liver cells in rats and mice (Derman *et al.*, 1981; Powell *et al.*, 1984). cDNAs were prepared that were complementary to mRNA that was specifically present in liver but not in spleen, brain, or kidney mRNA. Labeled RNA was prepared from isolated nuclei where engaged polymerase II molecules were allowed to elongate chains by 300–500 bases in the presence of labeled nucleotides. The "run-on" analyses showed that the primary transcripts of a dozen different mRNAs (for example, albumin mRNA and other serum proteins) were made in adult liver nuclei but in all cases were not made in brain, kidney, or spleen nuclei. Thus transcriptional control was exercised over a range of genes in specialized tissues, whereas other genes, those for actin and tubulin, were transcribed at similar rates in all cell types.

The upsurge in molecular genetics in the 1970s was matched by greatly simplified procedures for protein purification in the 1980s. We joined the burgeoning group of laboratories interested in transcription factors, our particular interest being transcription factors that might be responsible for, or at least assist in, causing adult liver cells to exhibit such a marked specific transcription pattern. Three technical advances in particular were responsible for success in identifying and purifying specific transcription factors. First was the remarkable work in Bill Rutter's lab (Edlund *et al.*, 1985). Short deoxynucleotide stretches upstream of initiating sites in several genes expressed specifically in the pancreas were, when appended to reporter genes and introduced into cells, capable of giving at least some cell-specific transcriptional direction. Second, such specific DNA stretches were capable of binding proteins in extracts from cells active in transcription of those particular genes, whereas other cells lacked such site-specific binding proteins. Finally, these deoxyribonucleotide stretches could be synthesized chemically and attached to solid substrates and then be used to purify specific transcription factors. Such purifications were carried out many dozens of times all over the world, including in the community of labs studying cell-specific transcription in the liver. The first of these proteins to be purified from liver by Steve McKnight and colleagues was called CEBP, because it bound to a C-rich site in viruses. These workers were not intent originally on studying liver-specific gene transcription (Johnson *et al.*, 1987). Gerald Crabtree and colleagues and Riccardo Cortese and colleagues purified and cloned a protein that became

known as hepatocyte nuclear factor 1, which was thought to have a role in serum protein formation (Courtois *et al.*, 1987; Frain *et al.*, 1989; Baumhueter *et al.*, 1990). Eseng Lai and Francey Sladek in our group also purified the proteins and cloned the genes for transcription factors that by binding assays on liver-specific promoters were only present at high levels in liver nuclei. Thus we identified HNF3α, β, and γ and also HNF4 (Lai *et al.*, 1990, 1991; Sladek *et al.*, 1990). All of these factors have proved to be important in early development or in liver function, but none is devoted exclusively to liver function. The collective conclusion of our work and that of the several other labs active in the same pursuit was illuminating if perhaps a bit disappointing. All of the regulatory regions of the genes transcribed specifically in liver nuclei bound at least several of the liver-enriched transcription factors. However, none of the factors was a dominant "master gene" for liver control (Sladek and Darnell, 1992). In addition, neither the order of binding nor the position relative to the start sites of transcription was conserved in the many different genes transcribed specifically in liver cells. Thus, although it was clear that the proteins we had uncovered were important in liver-specific transcription, each gene used a different combination of these liver-enriched proteins plus widely distributed transcription factors to accomplish the demonstrated cell-specific transcription. On reflection, this situation is only reasonable because the individual gene is the evolving entity throughout time, and the genes we studied because of liver-specific expression had origins earlier in evolution than the liver did. Thus we could have hardly expected a code in the transcription factors that spelled out "LIVER."

One final set of experiments on liver-specific transcription is useful to bring this discussion to a close. David Clayton, a graduate student, had showed the dependence of continuing liver-specific transcriptional pattern on cell–cell contact among hepatocytes and surrounding proteins (Clayton and Darnell, 1983; Clayton *et al.*, 1985). That is to say, when the cells were disaggregated they continued to form liver-specific proteins, but within a matter of a few hours they stopped making the primary transcripts that gave rise to the liver-specific mRNAs. These experiments led to the major theme that our lab has followed most actively over the last 15 years, namely, how cell surface contacts between proteins lead to specific gene transcription. Following this trail led to the discovery of the STAT (signal transducer and activator of transcription) proteins and the Jak-STAT pathway of transcription control. A recent summary (Darnell, 1998) has been given of the origin and progress of that work, which, however satisfying, does not need a second review here. That work does fit, however, as does everything summarized in this article, into the pursuit of one constant question over the years: How are human genes regulated? It has been, virtually on a daily basis, a thrilling and deeply satisfying life work, privileged from the beginning by working in Harry Eagle's lab and since then in some of the country's and world's outstanding research institutions. I count myself a most fortunate person. A final note is in order. More than 100 young people have joined me as students and postdoctoral fellows, and their productive years while in our laboratory coupled with their enormous success as scientists on their own is the source of my greatest pride.

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