Reminiscences on my life with RNA: a self-indulgent perspective

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In 1970 I came to MIT as a postdoc with Sheldon Penman. After my PhD in Developmental Biology studying chicken limb development, I was unsatisfied with phenomenology and decided that I wanted to get to the mechanics of cell differentiation. I chose the new field of molecular biology. I knew nothing about RNA, but thought it sounded like the molecule to work on if one was to pursue cell differentiation. The place for molecular biology was MIT and the place for RNA was Sheldon. Sheldon was kind enough to take me in, despite my lack of even the most rudimentary knowledge concerning nucleic acids. He kindly told me that he hoped I would contribute some valuable new perspective to the lab, but rather I was the one who received the new perspective. Sheldon was trained as a physicist and therefore had a strong quantitative bent, which fortunately I absorbed. He had used this to derive methods for extracting and characterizing RNA, initially as a postdoc with Jim Darnell. The first year was very painful for me, and I bungled my way through poorly executed experiments, and constant overdoses of P32 and phenol burns, something that Sheldon (and the others in the lab, such as Michael Rosbash) did not overlook. Sheldon's most popularly used expression was "Think clearly now!" Since we decided that my focus would be on messenger RNA, it was important to try to isolate it away from the overwhelming amount of ribosomal RNA. Sheldon advocated the use of drugs that would inhibit pol I transcription selectively, and I tried many of these that were just coming available from the NCI program to discover new drugs (camptothecin for instance), but still the doses required to suppress pol I also significantly inhibited pol II, and unfortunately the peak of mRNA in sucrose gradient centrifugation fell exactly under the 18S peak of the smaller of the ribosomal RNA peaks. Even with short pulses of tritiated uridine that favored the faster turning over mRNA and its richer U composition, ribosomal RNA still predominated. Aviv and Leder isolated globin mRNA during this time because it was small enough (9S) to separate from the ribosomal RNA peak (PNAS 1972). I despaired of ever characterizing general mRNA and considered going into the clergy, when a breakthrough came in the person of Rosbash bringing back information from visiting Darnell at Columbia, that Milton Adesnik in his lab (*Science* 1971) had discovered that mRNA had a 3′ stretch of poly A (Mary Edmonds and Joe Kates also discovered this), and a light bulb went off immediately in my mind that this was the way to isolate mRNA. Using poly U affixed to glass fiber filters using UV, I isolated the first mRNA from HeLa cells on Christmas Day 1971; the sedimentation was very heterogeneous, with a peak at 18s. The results were published in *Nature* in 1972 (at the same time Mary Edmonds also published her purification using oligo dT in *JBC* and Joe Kates showed it while studying vaccinia virus in *PNAS*). I went on to characterize the synthesis and decay kinetics of mRNA (*JMB* 1973).

Then followed a long period of time where I tried to develop an approach to relate RNA to cell biology. This came from my desire, formed during my PhD, to investigate gene expression related to differentiation. I had gone to Israel to work with David Yaffe on differentiating muscle in culture. We tied to determine the mRNAs that were turned on during development, but this was difficult before cloning. We tried to isolate myosin mRNA since it was expected to be so large that it would sediment faster than the other mRNAs. We were unable to identify it unequivocally. The problem was that differentiating cells do not do all the same thing at once, and therefore one could not pinpoint when exactly a gene was turned on using ensemble measurements with cell populations. Hence the differentiating cells turning on a gene were greatly diluted in the population.

I started my own lab at the new University of Massachusetts Medical School in 1974 at the age of 29 (things went more rapidly back then because there was less competition and the biological world was expanding rapidly). I saw clearly a method was necessary to study gene expression in individual cells, where the morphology could be correlated directly with expression. Hence I put my effort into single cell techniques, notably in situ hybridization. It was during this

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time that I came into contact with David Ward at Yale, who had developed a nucleoside analog containing a biotin moiety that, when incorporated into DNA, allowed isolation of viral sequences using avidin beads. I approached him about a collaboration because it was immediately clear to me that we could use it in cells to detect RNA directly, without the extraordinary effort that involved using isotopically labeled probes and autoradiography. We used it to show that actin mRNA could be detected in differentiating muscle. More years went by optimizing the technology using C14 thymidine, and H3 uridine labeled cells combined with a P32 labelled probe so that respectively cell number, RNA retention, and probe hybridization efficiency (nick translated probe from an actin clone provided by Don Cleveland) could all be monitored quantitatively for in situ hybridization. Many thousands of samples were assayed with permutations in many variables, and it wasn't until 1985 (*NAR*) that we had a robust protocol, one that is still used today. The most important contribution of this protocol, at least by the literature abundance, is FISH.

Having a protocol that allowed hybridization to intact cells where the RNA was quantitatively retained led me into using microscopy to achieve the unification of cellular and molecular biology for which I had been striving. This in turn has provided us with critical insights as to how the cell regulates RNA, in particular that mRNA localizes in particular compartments of the cell (Lawrence, *Cell* 1986), and that this is due to "zipcode" sequences most often in the 3′ UTRs, that are transduced by binding proteins into cellular spatial information (Kislauskis, *JCB* 1993). The localization of mRNA has been a major contribution of this line of work to the understanding of embryonic and cellular polarity, and ultimately what determines the morphology of cells. It will likely turn out to be one of the cellular bases of disease.

The microscope was not a common tool for molecular biologists, but fortunately I had some experience in my PhD. Capturing images on film turned out to be a challenge, given the rapid bleaching of the hybridized probes, and the horrible background from the fluorescent streptavidin used to detect biotinated probes. Many combinations were tried but the most important advance was the use of synthetic oligonucleotides (when synthesizers became available in the mid-'80s) chemically coupled to the synthetic probes. Fortunately there was a nucleic acid chemist in the lab, Krishan Taneja, who solved this problem. The oligonucleotide probes were controllable in their concentration and importantly in their size. Nick-translated probes were sticky, creating backgrounds from large, fluorescent molecules that looked like hybridization.

The next major advance was the availability of CCD cameras in the late '80s. A colleague and close friend, Fred Fay, introduced me to the advantages of digital imaging, and it completely changed our approach. (Fred tragically died suddenly of a heart attack in 1997.) I am lucky to have found Shailesh Shenoy, an engineering student at Worcester Polytechnic Institute who trained with Fred and me and has kept up the microscopy development in my lab for the last 20 years. His constancy and dedication has been essential for our success.

It took a while for us (with a graduate student, Andrea Femino) to realize that we had the capability to detect single molecules of mRNA by simply multiplexing many probes to the RNA template to increase the signal (Femino, *Science* 1998). The next major advance was the ability to detect mRNA in living cells by using the same multimerizing principle, a capsid protein from MS2 fused to GFP to bind to MS2 stem loops inserted into a reporter mRNA (Bertrand, *Mol Cell* 1998). This has opened a whole new world to the study of mRNA not possible with any other method since it does not involve perturbing the cells. The insertion of the MS2 cassette allows study of mRNA expression (Janicki, *Cell* 2004; Shav-Tal, *Science* 2004), in its native environment in animals (Park, *Science* 2014), in its transport of mRNA through nuclear pores (Grunwald, *Nature* 2010), in the expression of a single gene (Larson, *Science* 2011), in the regulation of mRNA at synapses (Buxbaum, *Science* 2014), in the translation of single mRNAs (Halstead, *Science* 2015). Much more will come from this technology as previously intractable biological questions involving RNA come within within our grasp.

It is intellectually satisfying to see how the study of RNA has evolved over the years, and the journal *RNA* came out of this as a natural development. I think that there will continue to be many years of discoveries about RNA that we can't anticipate, and the journal will continue to be the historical repository for these events.