We'll always have RNA

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My romance with RNA began in Paris at the age of 21. After four undergraduate years at Caltech and before going to graduate school at MIT, I somehow managed to spend a miraculous year in the city of light, where I worked in the laboratory of Marianne Grunberg-Manago. She had done a post-doc with Severo Ochoa at NYU, where she discovered the enzyme polynucleotide phosphorylase (PNPase). This was thought at the time to be the enzyme that synthesized RNA in bacteria, for which Ochoa was awarded a share of the 1959 Nobel Prize in Physiology and Medicine. Given that the physiological role of PNPase is to degrade RNA rather than synthesize it (the enzyme is reversible of course, and Grunberg-Manago and Ochoa studied the reverse reaction), this topic in Marianne's lab presaged my long interest in RNA processing. Marianne was also working on the regulation of bacterial protein synthesis when I arrived there in the fall of 1965. This was my topic during that Paris year, which anticipated my graduate studies at MIT. I worked there in Sheldon Penman's lab on eukaryotic protein synthesis as well as RNA.

MIT was truly the cat's meow. In addition to the magnificent Department of Biology with its remarkable faculty, graduate students and post-docs, my PhD lab was a wonderful local environment. It is difficult to convey in a few words how talented and enthusiastic Sheldon was, how much energy he put into mentoring his students and post-docs. The lab also had a gaggle of talented and entertaining people when I was there in the mid-late '60s and early '70s. (See article by Rob Singer.) I then went to Edinburgh where I worked on gene expression in the laboratory of J.O. Bishop, including a radioactive method to quantitate the poly A tails of mRNAs. The latter served me well when I began my own laboratory at Brandeis.

The "path to independence" as it is called today was more straightforward in the '60s and '70s; I did a 4.5-year PhD, spent six more months in the same lab as a post-doc before my three-year post-doc in Scotland, and I then started my faculty position at Brandeis at the age of 30. Almost all of my peers followed a similar path. This was mostly due to the circumstances of the era, namely, an expansion of life science depart-

ments and NIH-sponsored research across the US as well as the retirement of a cohort of faculty hired after WWII.

I was the beneficiary of a receptive and stimulating environment when I arrived at Brandeis in October of 1974, almost exactly 40 years ago. Most notable were the fields of yeast physiology and genetics (yeast genetics as well as fly genetics), neither of which I knew anything about. Although recombinant DNA and the more recent genomics era have fused the discipline of genetics with more molecular or biochemical approaches, my then Brandeis contemporaries were trained in classical genetics, Lynna Hereford in yeast genetics and Jeff Hall in fly genetics. Both of them had a profound influence on me professionally.

Lynna began yeast studies in my lab during the mid-'70s, which was the beginning of the recombinant DNA era. After an initial foray into genomic studies, she decided to focus on histone genes and their regulation, whereas I chose to study yeast ribosomal protein genes. It was known that most of these proteins were stoichiometric in the ribosome; how did this occur? Based on the fact that these genes were known to be present in operons in *E. coli*, everyone assumed that there would also be some coordinate regulation of yeast ribosomal protein (rp) transcription, reflecting perhaps some common genomic organization. Cloning these genes was the first task during these early days.

My attention was also drawn to an intriguing and ultimately invaluable set of yeast temperature-sensitive (ts) mutation strains originally identified by McLaughlin, Hartwell, and Warner. A shift to the nonpermissive temperature in most of these "RNA" mutants rapidly inhibited the synthesis of most rps at the restrictive temperature. As the original name "RNA" implies, the screen was designed to identify mutants that affected the synthesis of mRNA. However, the pulse of radioactive precursors was for one hour, much too long to avoid the dominance of rRNA incorporation. Once this was realized, the conclusion was that the mutants affected rRNA transcription rather than mRNA transcription. This also turned out to be incorrect, as it was the stability of newly synthesized rRNA rather than its transcription that was affected. Degradation of rRNA occurs almost immediately in

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the absence of an adequate supply of ribosomal proteins, the synthesis of which was rapidly and potently inhibited upon shifting to the nonpermissive temperature. So the interpretation shifted once again: the effect on ribosomal RNA was indirect, and the mutants directly affected the coordinate transcription of yeast rp mRNAs. As my lab was cloning rp genes, I set up northern blotting—a brand new technique at the time—to assay these mRNAs and in particular the response of their levels to a shift of the mutants to the nonpermissive temperature.

Indeed, the rp mRNA levels decreased rapidly in these mutant strains, but there was a major surprise: new higher molecular weight bands appeared upon the temperature shift. Taken together with some preliminary gene characterization, the data suggested that many yeast ribosomal protein genes contain introns. Only the actin gene had been shown to contain an intron by the Abelson lab, and it was unclear at the time how many more intron-containing genes existed in the yeast genome. Because of the increased levels of the higher molecular bands after the temperature shift, a second suggestion was that the RNA mutants did not affect transcription but affected intron removal and therefore encoded components of the splicing machinery. As a consequence the RNA mutants were renamed PRP mutants; PRP stands for pre-mRNA processing. Incredibly, it was once again RNA processing rather than transcription that won the day; the apparent specificity of the mutant strains for rRNA and rp mRNA was a consequence of the remarkably high fraction of yeast rp genes that contain an intron. This was my last first author experimental paper.

John Abelson was very excited by our new results and paid my lab a visit shortly after the paper appeared. His lab led the effort over the subsequent years to exploit these mutants to study splicing in vitro, whereas Christine Guthrie and many others made outstanding use of these and other yeast mutant

strategies to study splicing in vivo. My own lab continued to study yeast pre-mRNA splicing for the next 25 years.

Paris was once again relevant to this yeast effort, because of the many wonderful French students and post-docs that came to my lab over these years. That initial visit I made to Paris at the age of 21 in 1965–1966 continued to be influential as Marianne was a good pal of Piotr Slonimski. He was a yeast mitochondrial geneticist who worked in Gif-sur-Yvette just south of Paris, and his genetics work was important for an understanding of mitochondrial splicing. Piotr somehow knew who I was, presumably through splicing as well as through Marianne. He sent Brandeis and me an outstanding student, Claudio Pikielny, who had done his diploma work—an undergraduate senior thesis more or less—with Piotr. Claudio was the first in a series of wonderful French students and post-docs, who came to my lab. Whatever I learned about RNA was due to them and the many other students and post-docs who worked in my lab over the years.

As a coda, I should mention that my RNA work and background were highly relevant to my circadian rhythm work, which was ongoing at the same time. Two simple thoughts come to mind. First, the transcriptional feedback loop that underlies circadian timing could not have been discovered in my lab without the parallel effort on yeast RNA. (The two topics were physically intermingled within my single lab.) Knowing how to analyze RNA, specifically to do RNase protection assays in those pre-PCR days, was key. Second, my RNA background had taught me that most eukaryotic mRNAs as well as many proteins have half-lives of hours. Gene expression components with these kinds of time constants—as opposed to neuronal channels that operate in the millisecond range—was a very appealing foundation for a 24-hour clock. Many circadian biologists were schooled in neuroscience and ignorant about the wonders of RNA. More's the pity.

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