RNA

Life's Indispensable Molecule

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The Rockefeller University

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Front cover artwork: An RNA molecule morphing into a staircase, representing the historical progress of RNA research, electrifies the dawn sky, representing life. (Credit: Jean-François Podevin; © 2011 Photo Researchers, Inc.)

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To all my family—both the old and the new

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Preface

A SIZEABLE NUMBER OF COLLEAGUES READ all or portions of this book at various times during its preparation. Most believed, as I did, that a book stressing the history of those phases of molecular biology centered on RNA was a sound and different idea and should produce a useful, even a needed, book. Early discussions with Shai Shaham, Paul Nurse, Sid Strickland, and Jan Breslow helped particularly in shaping the content of what was finally included. My gratitude to each of them.

As the project developed, I received positive encouragement, advice, suggestions, and corrections from a larger group. That list includes David Allis, Jan Breslow, Linda Chaput, Gene Cordes, Bob Darnell, Ford Doolittle, Jeff Friedman, Magda Konarska, Leon Levintow, Peter Model, Tom Muir, Paul Nurse, Lennart Philipson, Bob Roeder, Marjorie Russel, Shai Shaham, Sid Strickland, Jon Warner, and Mike Young.

To all of those friends and colleagues I offer my sincerest gratitude. For the remaining errors and especially for the omitted or neglected references (despite ~1000 included references), the responsibility remains with me.

Initial discussions with John Inglis, the Executive Director of Cold Spring Harbor Laboratory Press, were extremely encouraging and helpful in solidifying the purpose of what is presented. The Cold Spring Harbor Laboratory Press has a staff exceptionally gifted in publishing scientific works, and I am eternally grateful to all of them: in particular, Inez Sialiano, Project Manager, and Rena Steuer, Production Editor, who among many other wise contributions arranged to make figures taken from older articles readable. I especially thank Maria Smit, the Developmental Editor. I've had considerable experience in biology textbook writing and publishing but have never had anything approaching the skillful and thoughtful editorial help I've received on this book. Lois Cousseau, my assistant for more than 30 years,

merits very special thanks. Nothing I've written in all this time, all beginning in longhand, would have ever appeared without Lois's cheerfulness, patience, and extraordinary competence. Even the most heartfelt thanks seem insufficient.

Finally, this labor of love was supported with my wife Kristin's unflagging confidence and belief that I could do it.

Author's Note

Pursuing RNA for More Than 50 Years

IF LENGTH OF SERVICE IN PURSUING the mysteries of RNA qualifies one as a reporter of RNA history, I suppose I easily qualify. As luck would have it, my earliest research after a medical internship directed me as a complete naïf toward studying RNA. Some personal events of those long ago years are given here.

I joined the laboratory of Harry Eagle at The National Institutes of Health (NIH) in Bethesda, Maryland, in July 1956, where he had just established the nutritional requirements for the growth of animal cells in culture (Eagle 1955). This landmark advance made growing homogeneous populations of animal cells in some ways comparable to growing uniform populations of bacterial cells and, of equal importance, provided uniformly susceptible cells with which to study animal virus replication.

Eagle had done one collaborative experiment with the virologist Karl Habel on nutritional requirements for poliovirus growth in HeLa cells (Eagle and Habel 1956) but had his hands full with other pursuits, particularly protein turnover. I did not know a virus from a billiard ball, and he could have directed me to work on any number of things. But it was my great good fortune that he suggested I work on virus infection in cultured animal cells, and he assigned me a bench in a room with Robert I. DeMars, Salvador Luria's third graduate student. Luria was, of course, one of the founders of modern bacteriophage research and the molecular biology that grew out of that research. DeMars handed me his copy of Luria's 1953 text *General Virology*, the first successful textbook ever written exclusively about viruses and, incidentally, according to Luria, the first textbook to describe the Watson-Crick structure of DNA (Watson was Luria's first graduate student). DeMars said

to me, "Read this and I'll teach you virology." Thus, under DeMars's tutelage, I followed somewhat in lockstep what a phage worker would do, but with an animal virus. I established a plaque assay for the RNA-containing poliovirus (Schaffer and Schwerdt 1956) and showed that every HeLa cell could be infected and yield virus (Darnell 1958).

Together with my colleague, mentor, and close friend Leon Levintow (Levintow and Darnell 1960), we developed a simple purification scheme for poliovirus using CsCl density centrifugation and then, using labeled nucleosides and amino acids, determined the time course of formation of poliovirus protein and poliovirus RNA (Darnell and Levintow 1960; Darnell et al. 1961). The RNA was formed first, as befitted the director of protein synthesis; Heinz Fraenkel-Conrat and colleagues had also shown this to be true for tobacco mosaic virus (Fraenkel-Conrat et al. 1957). Moreover, as had been done by this time with other animal viruses (Colter et al. 1957; Wecker 1959) (and, of course, plant viruses [Gierer and Schramm 1956]), Levintow and I found that the phenol-extracted RNA from poliovirus and from poliovirus-infected cells was infectious (Darnell et al. 1961).

These experiments did not signal any important contribution of ours about how RNA could exert its protein-specifying role. However, this work did earn me a stay in Paris in 1960–1961 as a postdoctoral fellow at the Pasteur Institute with François Jacob in the Service de Physiologie Microbienne directed by André Lwoff. The sojourn in Paris could not have come at a more propitious time for a young researcher interested in the actions of RNA.

When I climbed up to the famous third-floor laboratory in the last week of August 1960, I went into the office and found Sarah Rapkine, Lwoff's assistant, who had rented an apartment for us. She enquired solicitously about our well being (my wife Jane and three little boys were along for our Paris adventure), and seeing that we were at least getting along, she brought me in to meet André Lwoff. After a perfunctory but pleasant conversation and an introduction to Marguerite Lwoff, André's wife and collaborator, André introduced me to François and to Elie Wollman. Within the space of no more than one or two incorrectly worded sentences by me, François announced, smilingly, that we would speak English. Perhaps, for a few milliseconds, my brain thought to be embarrassed at my incompetent unworldliness, but relief instantly overwhelmed pride.

François knew that I had done a little biochemistry with polio-infected HeLa cells, including the use of zonal and density gradient equilibrium sedimentation. We struck up a bargain. He would speak genetics slowly enough for me to get an inkling of what was going on in his experiments, and I would try some "molecular biology" and teach everyone who wanted to know how to make and separate RNA on sucrose gradients. Many

geneticists were gearing up to study the newly described messenger RNA (mRNA), as I was to learn.

Although the expression "molecular biology" was still fairly new, I had gotten the drift at NIH that that was what I was doing with polio. But it was clear that I needed badly to catch up on what had been published from Paris. For example, Jacob told me to really understand the experiments that by then were referred to even in Paris as the PaJaMa (Pardee, Jacob, Monod) experiments. (They had been published in 1959 in Vol. 1 of the *Journal of Molecular Biology* [Pardee et al. 1959].) And a few days later (about mid September), François handed me a draft of "the" paper—"Genetic Regulatory Mechanisms in the Synthesis of Proteins" by himself and Jacques Monod (Jacob and Monod 1961). This paper was to become, next to the Watson-Crick papers of 1953, perhaps the most famous paper in molecular biology. He also told me of what he and Sydney Brenner (with Matt Meselson's help) had done at The California Institute of Technology (Caltech) in the spring of 1960 and had not yet published. Later that fall, François showed me the manuscript that he and Sydney had finished that proved the existence of the bacteriophage T4 mRNA. Thus, not only did I get to read before publication the logic behind the idea of a "messenger" (Jacob and Monod 1961) but also the best experiment to establish the existence of mRNA (Brenner et al. 1961). All of the science underlying these conclusions is found in Chapter 2.

Here I was, just turned 30, having done some journeyman work on an RNA-containing animal virus (polio) and having landed in one of only three places on the planet (The Pasteur, the Watson laboratory at Harvard, and Sydney Brenner's laboratory in Cambridge) that knew the secret of information transfer from gene to protein. Now, 50 years later, not a day passes that I don't remember and reflect on my luck as a youngster just getting started.

These early experiences sent me on my way to study RNA for all of the 50 plus years of my scientific career. Whether this long experience equipped and entitled me to be a historian of RNA, we must let the reader decide.

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