From exotic to exciting

ECKHARD JANKOWSKY
Center for RNA Molecular Biology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106-4973, USA

The last 20 years coincide almost perfectly with the time I have been working on RNA. I would therefore like to take a very personal view over that time and abstain from talking about specific results of either our work or the many insights from others that have impacted our field—RNA helicases. The science is frequently discussed in reviews.

What often goes unnoted are opportunities brought by new methods or equipment. In fact, it was a piece of equipment that motivated me to work on RNA. In 1993 I had started my PhD studies at the Chemistry Department of the Technical University Dresden, at the newly minted Biochemical Institute. Dresden is in the former East Germany, and at this time the political adjustments in Germany were still ongoing. There was not a lot of money to go around to equip the new institute with both people and instruments. However, there was a brand-new ABI DNA/RNA synthesizer. I made it my mission to use the machine for something exotic—to make catalytic RNA-hammerhead ribozymes, and to study them. For me as a chemist it was intriguing to work with a biological molecule made from scratch just by chemicals. Working on RNA was also exciting, because then, as now, something profoundly new about RNA was discovered all the time.

In early 1997 I got the chance to move to New York City to postdoc in Anna Marie Pyle's lab at Columbia University. This was eye opening. Jennifer Doudna came to talk about her new P4/P6 structure. At meetings I saw the first structures of RNA–protein complexes that we now teach as classics. And I got a glimpse of RNA biology. In Anna's lab, I started to work on RNA helicases. By that time, it was clear that these enzymes were ubiquitous, and conserved. The "Birth of the DEAD-box" (Linder et al., *Nature* **337**, p.121, 1989) had been announced, and we were "… alive with DEAD-proteins" (Wassarman and Steitz, *Nature* **349**, p.463, 1991). There was much excitement about RNA helicases, but it was not understood how these proteins worked on an enzymatic level. I liked starting from scratch, and we developed ways to define basic biochemical features of RNA helicases, focusing on a viral enzyme. We did learn quite a bit about the enzyme including the fact that it could remove proteins from RNA. We also learned that the reactions usually involved many steps, some of which were important but simply invisible with traditional biochemical and biophysical approaches.

Just at the right time, a new method had emerged that allowed one to look at individual enzymes at the single molecule level. With these new approaches one could measure even short, normally invisible steps, and one could see the sequence in which the steps occur. For an enzymologist with interest in multi-step reactions, this is a quantum jump from traditional ensemble methods. I went to learn single molecule FRET in Steven Chu's lab in Stanford, where his postdoc, Taekjip Ha had built a machine that a mortal biochemist could use and possibly build. Literally seeing your enzyme work on the single molecule level is profound, at least for the first time. In the single molecule FRET technique that I used, one sees little fluorescent spots blinking. "Seeing" your enzyme work is special.

In early 2002, I started my own group at Case Western Reserve University. Here, I have come to appreciate the many facets of RNA biology, and it seems that progress in RNA biology is accelerating each year. Looking back on how our work now differs from that of 13 years ago—the changes have been profound. For our studies on RNA–protein interactions and on RNA helicases, the biggest change was probably brought by computational power, something that has little to do with the RNA field itself. Our early work focused on one or at most few reactions of a single enzyme. Now we study multi-component complexes, and routinely work with models that encompass dozens of reactions, and sometimes hundreds of rate constants. The computational models have become an integral part of the studies. There is constant back and forth between experiment and model. But all computation does not substitute for the need to understand individual steps, and for the need to produce good samples. Models have to be evaluated very critically, and this is often not possible on an intuitive level. The learning curve for new students is much steeper and longer than a decade ago. While this is hard, it is also exciting. I would have never imagined, even 10 years ago, to quantitatively describe enzymatic systems with three or more components.

Corresponding author: exj13@case.edu
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The second, and perhaps most profound change for our work is next generation sequencing (NGS). As noted, I started out chemically synthesizing all of my RNA and DNA reagents, so thinking in cellular terms is a big step. Yet, the perspective to quantitatively look at cellular processes that involve RNA, and to study "our" enzymes quantitatively in the cell, was just too intriguing to pass on. Our projects on this front are exciting because they chart completely new territory, at least for us. For example, NGS allows us to measure rate constants for thousands of different RNAs simultaneously, and this has helped us to much better understand what it means for an RNA-binding protein to be specific—or nonspecific. In the hopefully not too distant future, we think we will be able to eliminate the divide between biochemical and biological studies for the systems we are studying. Regardless of when we will get there, I find it incredibly motivating that it now appears perfectly reasonable to strategize how to measure exactly the same reaction steps, in vitro and in the cell for all RNAs.

Working on RNA is perhaps less exotic than it was 20 years ago, but it is much more exciting, because now we can do what we might not even have dreamed of in 1995.

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