
RNA dances to center stage

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It has always been about RNA. In 1983, I was a senior at Johns Hopkins studying chemistry, and flailing about as one of the worst practicing organic chemists in history. Fortunately, I took classes with Warner Love and David Draper that opened my eyes to the world of biophysical investigation of macromolecule structure and function, in particular of RNA. This was a special time for RNA science, with the discoveries of Cech and Altman of RNA catalysis. My desire to mix chemistry and RNA was piqued.

The key challenge in 1984, my first year in graduate school with Nacho Tinoco at Berkeley, was to understand the structure of RNAs. At that time, there were only several crystal structures of tRNAs and a couple of small oligonucleotides. How could we explain structurally the newly discovered catalytic functions of RNA? The structures of tRNAs suggested complex folds, but clearly could not represent the full repertoire of RNA structures. We needed access to the molecular complexity of RNA.

Science advances through a confluence of technology and serendipity. Fortunately, several major advances in the 1980s and 90s would change our views of RNA structures. Structural and biophysical studies require large amounts of pure, homogeneous macromolecules. Whereas protein overexpression was revolutionizing protein structural work, obviating the need for laborious purifications for natural sources, there was no such system to make RNAs. In the mid-1980s, Uhlenbeck and co-workers harnessed a simple phage T7 RNA polymerase to allow *in vitro* transcription of any defined RNA. Finally, milligram quantities of RNAs could be prepared and purified for structural investigations. This advance was in turn propelled by the development of chemical DNA synthesis to create defined templates. Unfortunately for our large egos, breakthroughs are built on the shoulders of technologies.

Nuclear magnetic resonance (NMR) spectroscopy underwent a transformation in the 1980s as well, powering its use in biophysics. Stronger magnets using superconducting materials meant higher sensitivity and spectral dispersion, allowing access of the chemical approach to complex biochemical systems. The development of multidimensional spectro-

scopic experiments alleviated problems of spectral overlap in large macromolecules, and allowed robust correlations among nuclear spins that are required for structural analysis.

The application of NMR to RNA in the 1980s flowed naturally from these developments. To address the limited structural data on RNA, samples could be prepared at a milligram scale and structural data could be extracted using NMR. Early experiments showed how RNA hairpins, internal loops, and pseudoknots folded, yielding unprecedented insights into the folds of RNA elements. In the early 1990s, Williamson, Pardi, Varani and Feigon, among others, pioneered the use of stable-isotope labeling, and development of heteronuclear, multidimensional NMR. This development led to true NMR structure determinations on multiple systems, extending into structures of RNA–protein complexes. The impact of NMR on our understanding of RNA structure was perhaps even greater than its impact on protein structure.

The power of X-ray crystallography to determine structure is unparalleled. Soon, clever crystallographers, led by Dave McKay and Jennifer Doudna, broke the long drought in RNA structure by crystallography. The structures of the early 1990s revealed how ribozymes folded, how catalysis might occur, and most importantly how large RNAs might pack their helical elements into a compact fold. This golden age of crystallography was driven again by improved crystallization approaches, vast improvements in synchrotron radiation and computational tools, and culminated in the extraordinary ribosome structures of 2000; it continues unabated today.

Technologies upend accepted notions. In the mid 1990s, NMR was an equal, albeit less powerful, contributor to structural investigations of RNA. NMR remains a powerful approach to probe structure and dynamics of RNAs and RNA–protein complexes, but crystallography on large assemblies became the norm. My own interest was always in the conformational dynamics of RNAs and how ligands could modulate those changes. Luckily, another set of technologies and circumstances came along to tackle these problems.

Single-molecule approaches were revolutionizing biophysics in the 1990s and early 2000s. Single molecules could be manipulated with optical traps to measure biological forces, and improvements in camera and lasers allowed detection

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of fluorescence from single fluorophores in real time with millisecond time resolution. The key question was how dynamics of systems were coupled to function—single-molecule approaches allowed investigators to track biological processes directly as they happen, mapping conformational pathways using fluorescence resonance energy transfer (FRET). I moved to Stanford in 1997, which was perhaps the center for creative single-molecule work. Even more fortunately smart postdocs in Steve Chu's lab (TJ Ha, Xiaowei Zhuang) were creating the core technologies for single-molecule FRET, and they convinced Scott Blanchard, a graduate student in my lab, to try single-molecule fluorescence on the ribosome. He was joined by another student with Steve Chu—Harold Kim—and then a postdoc, Ruben Gonzalez. This remarkable team created a system to measure dynamics of tRNA selection by the ribosome, showing how single-molecule methods could be applied to complex biological systems. It was the start of 15-year voyage that continues still.

Static structures must be animated to understand biology. Dynamics and linkage to conformation and composition have been a focus of our research over the past decades. The themes are echoed from the discussion of NMR and crystallography—creating samples for single-molecule study, improved instrumentation and detection approaches, faster and more robust computation. These advances have led to the maturation of the field of single-molecule biophysics and its application to RNA—simple RNA structures, catalytic RNAs, riboswitches, RNA–protein complexes, the ribosome and spliceosome. These studies have revealed conformational pathways of folding and assembly, and the dynamics that underlie catalytic processes.

The future holds many challenges for single-molecule investigation of RNAs. One is expanding investigations to more complex eukaryotic systems. The underlying biochemistry and labeling of reagents represents a challenge for study of these systems. But new technologies are revolutionizing the investigation of human systems using CRISPR approaches. For example, we have recently labeled human ribosomes and studied initiation events involved in Hepatitis C virus infection. Perhaps these approaches will allow direct observation of eukaryotic translation.

The example of translation is illuminating. There have been huge advances in unraveling the complex biology of translation—including using ribosomal profiling with deep sequencing to get snapshots of translation. However, we must observe dynamic processes directly to parse pathways and regulatory branchpoints. This is a key challenge for the future. The assemblies and intermediates of translation in humans are often too heterogeneous and unstable for crystallographic methods. Yet, cryoelectron microscopy has itself been revolutionized by improved direct electron detectors, allowing atomic resolution studies of large systems. The merging of single-molecule dynamics and single-particle structures will be a step towards creating atomic-level molecular movies.

Eventually, we must understand structure and dynamics of RNAs *in vivo*. Sub-diffraction imaging methods are opening unparalleled real-time vistas onto biomolecular behavior *in vivo*. Mass spectrometry and sequencing have exquisite single-cell sensitivity and resolution. Breakthroughs will come through the continued interweaving of technologies. As in the past, it will be a thrilling and surprising voyage.