On the shoulders of giants

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Isaac Newton popularized the saying that we see further by standing on the shoulders of giants. As valid as this is, it has been my experience that how and in which directions we choose to look are most strongly influenced by mentors during our formative years. I had the privilege to be introduced to the scientific method and to experimental science during childhood by two formidable mentors. The first was my father (Ricardo Ferré-D'Amaré), an engineer turned anthropologist, who starting with my curiosity about some seashells I found on a vacation at the beach, taught me about the Linnean system. From there, he deftly guided my growing interest in malacology to an early passion for all things mollusk, and eventually, to marine biology and ecology. My other early mentor was Ricardo Klimek, a distinguished chemical oceanographer and limnologist, who taught me about experimentation in the physical sciences. On one of the secondary-school summers I spent in his field lab in a highland lake in Western Mexico, I managed to break his silver chloride electrode. He was an unflappable man, and his response was for us to drive to the silversmith in the village, purchase a length of silver wire, and manufacture a home-made electrode that was nearly as good as the commercial one.

Those early experiences set me on the scientific path. But I also had a mentor to whom I can directly trace my interest in RNA. One of my high-school biology teachers was Adelaida Sarukhán, who introduced us to the macromolecules of life in her freshman Biology class. I still vividly recall learning about how proteins do all cellular catalysis and how Watson–Crick complementarity and base triples in DNA code for proteins. Miss Sarukhán mentioned in passing that there was “another kind” of nucleic acid called RNA, and that it came in three flavors (mRNA, rRNA, and tRNA). She explained the roles of mRNA and tRNA in the flow of genetic information, and told us that not much was known about rRNA, that it likely helped ribosomes fold, but was not otherwise very important. That would have been a fine introduction at that level, but Miss Sarukhán had an inquiring mind. In that spring of 1983, she read about Tom Cech's discovery of catalytic RNA in *Scientific American*. In class, she asked us

to go back to our notes on RNA, and to add that, albeit probably just the exception that proves the rule, now one example of catalytic RNA had been recorded.

By the time I was a graduate student, Harry Noller had provided strong evidence that not only is rRNA important, it is likely catalytic, and Jack Szostak had shown how one can find RNAs with previously unknown catalytic functions in pools of random sequence RNA. It seemed to me, upon clambering onto the shoulders of those (and other) giants, that all that remained to be done on this subject was to figure out what RNA active sites look like, and how they perform catalysis. That quest has occupied many of us, RNA structural biologists and RNA enzymologists, for the last 20 years, and there is no end in sight.

Since that is the intention of this series, I will illustrate the excitement and camaraderie of the quest with four highlights from my experience. In 1998, while I was a post-doctoral fellow in Jennifer Doudna's group, I solved the crystal structure of the hepatitis delta virus (HDV) ribozyme. This provided the first glimpse of an enclosed, protein enzyme-like active site made of RNA. Most excitingly, I found that a cytosine residue essential for catalysis lay buried inside the active site pocket, looking provocatively like one of the catalytic histidines of the canonical nuclease protein RNase A. Since the structure I solved did not have bound substrate or inhibitor, the precise function of this cytosine was not immediately clear, but in a matter of months, our collaborator Mike Been biochemically implicated it in general acid-base catalysis. In the next few years, the Bevilacqua and Piccirilli labs provided definitive evidence of a general acid catalytic role for this cytosine. In a handful of years, this combined structural and biochemical work completed a paradigm shift, from the pre-HDV view that catalytic RNAs are all metalloribozymes, to the realization that, just like there are metalloenzymes and metal-ion independent protein enzymes, RNA has access to diverse catalytic strategies.

By 2002, the structural question was no longer what ribozyme active sites look like in the ground state, but when they are actually performing catalysis. Peter Rupert, the first post-doc to join my lab, succeeded in getting crystals of the hairpin ribozyme bound to a transition-state mimic. We

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had previously solved structures of this catalytic RNA bound to substrate and to products, and when we compared the three structures, we discovered that the RNA active site had evolved to maximize hydrogen-bonding interactions with the transition state. I learned in college that Linus Pauling had postulated selective stabilization of the transition state as the underlying principle for enzymatic catalysis, and here it was, another prediction from a giant confirmed.

Most protein enzymes use prosthetic groups and coenzymes. Can ribozymes do the same? This would not just expand the chemical repertoire of ribozymes, but also have important evolutionary implications, as Harold White had noted in 1976. The discovery of the small-molecule dependent *glmS* ribozyme by the Breaker lab spurred Daniel Klein to join my group as a post-doc with the intention of establishing whether the small molecule acted, in this case, as an allosteric activator or as a coenzyme. Daniel duly solved crystal structures of this RNA in multiple functional states, allowing him in 2006 not only to conclude that the small molecule is a true coenzyme of the *glmS* ribozyme, but also to propose a specific catalytic role for one of its functional groups. Subsequent work by the Fedor, Soukup, Strobel

and our labs, among others, confirmed his interpretation of the structures. Thus, in eight short years we had gone from not knowing what a ribozyme active site looks like, to visualizing catalytic RNAs positioning nucleotides, substrates, and even coenzyme functional groups with exquisite specificity. Indeed, in 2014, my graduate student Katherine Warner (and independently, Joe Piccirilli's group) reported crystal structures of the fluorogenic "Spinach" RNA (discovered in the Jaffrey lab), which not only binds its cognate chromophore with high specificity, but also manages precisely to control its electronic properties to induce fluorescence from an otherwise non-fluorescent compound.

Where next? Seeing is believing, but not necessarily understanding. To further the reductionist program, we need to dissect ribozyme catalysis in much grater detail. For instance, where are the hydrogen atoms and what are the ionization states of all active site moieties? On the biological side of things, surely there are many more RNAs with remarkable biochemical properties lurking in the dark matter of cellular RNA. I am certain that the most exciting discoveries are yet to come, and that they lie beyond bifurcations in our paths that we cannot envision yet.

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