Preface

The Discovery of Ribonuclease P and Enzymatic Activity of Its RNA Subunit

Sydney Brenner and Francis H. C. Crick had a specific project in mind when they offered Sidney Altman a position in their group in 1969 to conduct postdoctoral research at the Medical Research Council Laboratory of Molecular Biology (LMB) in Cambridge, England. At the time, an intense international competition was ongoing in as many as a dozen labs to determine the three-dimensional structure of tRNA. At the LMB, Aaron Klug was attacking the structure by crystallographic analysis with Brian F. C. Clark providing large amounts of purified phenylalanine tRNA. (Eventually, Aaron announced his empirically determined 3-D structure of yeast phenylalanine tRNA, a structure that is generally common to tRNAs, due in part to several conserved, novel three-way nucleotide interactions.) Concurrently, Michael Levitt, a Ph. D. student of Francis, was visually scrutinizing the cloverleaf secondary structure of the 14 tRNA sequences known at the time. Levitt was searching for nucleotide covariation in different parts of the molecules that were conserved in the 14 sequences known at the time. He identified a possible covariation of an apparent Watson-Crick pairing type between the residues at position 15 from the 5’ end of the tRNA and residue 48. This association implied these parts of the tRNA, namely the D loop containing residue 15 and the 5’ end of the T stem-adjoining residue 48, folded on one another in a tertiary structure shared by different tRNAs. In presenting this finding to Francis, Michael was concerned because Holley’s alanine tRNA sequence did not fit this covariation. Without skipping a beat, Francis quipped, ‘Well, of course, Bob Holley’s [Nobel-winning] sequence is wrong!’ a retort subsequently confirmed by Guy Dirheimer and associates.

Sydney and Francis, always reaching beyond the forefront of experimental methods and systems, wanted Sid to attack the 3-D structure of tRNA using nuclear magnetic resonance methods, which had not yet been applied to an RNA macromolecule. It had not escaped their notice that Sid had taken an undergraduate physics degree at the Massachusetts Institute of Technology, and had written his senior thesis in nuclear physics. However, when Sid arrived at the LMB and met Sydney and Francis, they told him explicitly that tRNA structure analysis by NMR was no longer necessary, and they suggested him to go away and think of a project on his own. Sid, politely scared out of his mind, went home and thought about a new project for 2 weeks and then met again with his mentors. This change in research direction was the first committed step toward Sid’s discovery of precursor tRNA, ribonuclease P, its RNA-protein composition, the enzymatic activity of the RNA,
Sid’s initial work at the LMB involved obtaining a tyrosine tRNA mutant that produced little mature tRNA, but when RNA was isolated by a new, rapid method, the mutant accumulated a metabolically unstable larger RNA, as indicated by its slower mobility during denaturing polyacrylamide gel electrophoresis. Next, Sid and John D. Smith demonstrated that the larger RNA was the mutant precursor tRNA by base sequence determination and by its site-specific cleavage at the 5’ end of mature tRNA by a nuclease (later named ribonuclease P) present in cell-free extracts. Others readily accepted these straightforward observations; in fact, within the week I had confirmed the site-specific cleavage by RNase P of T4 phage precursor tRNAs. In retrospect, the existence of a precursor tRNA was implied when Robert W. Holley determined the first tRNA sequence, for it contained a monophosphate at its 5’ end, whereas primary transcripts contain triphosphate residues.

Despite the novelty of Sid’s research, the Anna Fuller Fund fellowship supporting his work at the LMB was about to expire, just as he was embarking on the purification of RNase P activity. Recognizing the potential novelty and importance of Sid’s work (and his diligent work ethic), Sydney Brenner orchestrated a fresh fellowship for Sid to continue his work at the LMB. For the purification, Sid joined forces with Hugh D. Robertson whose expertise included nuclease purification. Sid’s last paper from the LMB describing the purification and properties of RNase P was published in *The Journal of Biological Chemistry*. The Discussion of this paper contained a provocative, terse sentence. While the statement was not especially noteworthy, and perhaps underappreciated by Sid, but not by his perspicacious coauthor, Hugh, its significance would eventually generate much ridicule and consternation, not only between Sid and his future students, but also vehement opposition from a number of senior scientists in other laboratories involved in characterizing the enzyme and among many biochemists in general. The pertinent sentence reads, “In light of these [purification] properties, it is possible that the active form of RNase P, which must have a strong negative charge, could be associated with some nucleic acid.” This sentence set the stage for a paradigm shift in defining the allowable chemical composition of enzymes.

Within 6 months, the enzyme had been substantially purified, but not freed of its nucleic acid component. Sid departed the LMB to accept a faculty position at Yale University and establish his own laboratory. One of his many gifted graduate students, Benjamin C. Stark, undertook further purification of RNase P to achieve homogeneity. Despite committed and rigorous work, Ben was unable to remove RNA from the active enzyme. Frustration and disappointment mounted at many levels within and outside the lab. Sid’s major extramural research grant was in serious jeopardy, as was his promotion to Professor. Furthermore, Ben became concerned about the content and sufficiency of his work for the Ph.D. degree.

Ben was nevertheless convinced that one or more RNAs of a specific molecular weight(s) were part of the enzyme. He repeatedly lamented to fellow graduate student, Sheldon I. Feinstein, that Sid was publically critical of his heretical proposal regarding the RNA. After hearing enough of Ben’s grieving, Sheldon told Ben to request a meeting of his Ph. D. committee to convince them that he should devise experiments to test
his radical idea. At the meeting, Sid and Donald M. Crothers asked Ben the pivotal question: how would he go about proving his proposal? Ben had an idea, but it was not a very good one. He would determine if pre-treating RNase P preparations with a ribonuclease degraded RNase P activity. The flaw with this approach was to assay the treated RNase P that would require a tRNA precursor substrate, and that an inevitable carry over of a miniscule amount of the degrading ribonuclease would abolish the precursor substrate. Peter M. M. Rae, another committee member, suggested that Ben pre-treat RNase P with a calcium-dependent microccocal nuclease, dialyze out the calcium from the digestion mix, and then assay RNase P. The latter enzyme requires magnesium but not calcium for its activity. Indeed, microccocal nuclease treatment abolished RNase P activity, demonstrating that the RNA component of the enzyme is essential for the reaction. These findings were published in the *Proceedings of the National Academy of Sciences, U.S.A.* in 1978, with Ben Stark as the lead author.

Subsequently, Sid and lab members designed a series of biochemical experiments to further characterize the RNA component of the enzyme.

One of several possibilities for the function of the RNA was to align the enzyme on the substrate tRNA precursor by interactions with nucleotides common to most tRNAs, which insured site-specific cleavage. (However, “The nucleolytic activity of the enzyme [was] reserved for the protein moiety.”) Perhaps, the tRNA segment of the precursor was folded into the conserved structure of mature tRNA, which the enzyme recognized. Searching the precursor-specific sequences in other tRNA precursors did not reveal an obvious consensus sequence for traditional base pairing. Another possibility, loosely derived from P22 phage biology, was the RNA served as a scaffolding structure, in this case centered on RNA, to configure the protein in its catalytically active conformation.

Another of Sid’s respected students, Ryszard Kole, was the lead author of the next *PNAS* paper, published in 1979, describing the dissociation of highly purified RNase P into component RNA and protein fractions. While each subunit lacked significant enzyme activity when assayed in the typical RNase P buffer containing a low Mg$^{2+}$ concentration (10 mM), mixing equal molar proportions of the two reconstituted activity.

Subsequent definitive experiments, published in *Cell* in 1983, demonstrated that the RNase P RNA component of both *Escherichia coli* and *Bacillus subtilis* contains the enzymatic subunit of RNase P, and that activity was not detected in the protein subunit. A senior associate in Sid’s lab, Dr. Cecilia Guerrier-Takada made this observation and was the lead author on the subsequent *Cell* paper. The activity of the RNA was maximal in a buffer that contained high Mg$^{2+}$ concentration (50 mM), unlike the normal 10 mM concentration in the buffer typically employed for assay of the intact enzyme. Serendipity prevailed in these experiments as the RNA-only experiment was intended as a negative control. When this control manifested cleavage, Cecilia suspected a simple accident had been made in the experiment, possibly by adding the protein component to the RNA-only sample. However, repeating the RNA-only experiment gave the same remarkable result! Collaborator Norman R. Pace, working at the University of Colorado Medical Center in Denver, had provided partially purified *B. subtilis* RNase P subunits for these experiments.
This experiment had been designed to test the activity of interspecies reconstituted RNase P, not RNA-only cleavage. Nevertheless, Sid offered to include Norm and his graduate student as coauthors of the Guerrier-Takada et al. (1983) *Cell* paper.

During this time, diverse and important factors contributed to Sid’s ability to maintain his work ethic and sanity. They included the strong support he received from his students, Yale faculty colleagues, particularly renowned biochemists, and other associates. The most enduring support, however, was the love of his family.

Following the difficult if not epic beginnings of research on ribonuclease P, the chapters that follow in this book describe the rich repertoire of basic and applied work to dissect, define, and exploit possible biomedical applications of the enzyme.

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*The author’s post-doctoral tenure with Sydney Brenner and Francis Crick coincided with those of Sidney Altman and Hugh Robertson.*
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