Reflections on the 20th anniversary of *RNA*

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What makes the ribosome tick? Proteins, proteins, proteins, it always seemed to be proteins, ribosomal proteins and a series of nonribosomal protein factors in particular. When I was cutting my molecular biology teeth, excitement centered on the identification, enumeration and characterization of the several dozen bacterial ribosomal proteins by column chromatography, gel electrophoresis, sequencing and biochemical analysis. How many were there? Were there sequences in common? Did they have enzymatic activity? Was one of them, perhaps, peptidyl transferase—the activity responsible for synthesis of the peptide bond? At the time, the role of ribosomal RNA was something of a mystery despite the fact that it comprised two-thirds of the bacterial ribosome mass. The analytical tools required to answer this question were few and far between.

It was established early on that rRNAs were not informational: they were uniform in structure and encoded no proteins. Could they then just serve as a scaffold for the ribosomal proteins? This didn't make much sense: it seemed that Nature wouldn't squander its resources on the synthesis of these giant molecules unless there were a more important reason for it. The first clues that rRNA might play a functional role in protein synthesis came from chemical modification studies in Noller's lab in the 1970s. Throughout the 1980s and 1990s, we slowly came to realize that the ribosome, despite its rather overwhelming size and complexity, carried out protein synthesis through a synergistic effort between RNA and protein.

Several decades ago, I, along with my colleagues, became fascinated by the process of ribosome assembly and, in particular, the manner in which ribosomal proteins interacted with rRNA. Nomura's lab had shown that the 21 proteins of the bacterial 30S subunit assemble on the 16S rRNA in a roughly sequential fashion, leading to the celebrated assembly map. One-third of the proteins bound independently and tightly to specific sites in the rRNA in the absence of other proteins. Once this group of proteins bound, other proteins then interacted with the nascent ribonucleoprotein, dependent on both the RNA and the presence of the primary binders. The lattice of interactions that defined the assembly

pathway was highly cooperative and clearly involved significant conformational changes in the rRNA before the subunit attained its final, compact structure.

The isolation of specific ribosomal protein–rRNA complexes, in conjunction with the development of RNA sequencing methodology allowed us to characterize the protein binding sites in the RNA. The expectation was that by determining the sequence and structure of enough protein binding sites, some sort of structural “code” would emerge that would reveal a common basis for these interactions. But that was not to be: it was recognized that each r-protein–rRNA interaction involved its own set of specific sequences, loops, bulges, pseudoknots and other special structural features of the rRNA. These molecules therefore proved to be very crafty in the way they carried out their scaffolding tasks and it soon became clear that this structural plasticity was intimately related to ribosome function.

The identification of sequences and DNA-like helices at ribosomal protein binding sites was fine as far as it went, but the real essence of RNA function was the versatility with which these molecules could form previously unanticipated three-dimensional structure. This lesson was learned from the crystal structure of tRNA in the 1970s, but didn't really penetrate the ribosome field until the structure of rRNA in the ribosome was elucidated by x-ray crystallography some 25 years later. Nonetheless, a growing appreciation of the importance of rRNA in ribosome function emerged from diverse genetic, biochemical and biophysical experiments. The work of my lab on tRNA-ribosome cross-links using tRNA containing single photoreactive nucleotides showed that tRNA was in very close contact with both ribosomal RNA and protein at the decoding site of the 30S subunit and the peptidyl transferase center of the 50S subunit. It was hard to escape the conclusion that ribosome function resulted from collaboration between these two classes of molecule.

In the end, high-resolution crystal structures from the Noller, Steitz, Yonath and Ramakrishnan laboratories that first appeared 15 years ago revealed the remarkable ways in which ribosomal RNA contributes to ribosome function as well as to structure. The basis of many different protein–

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RNA interactions—some of which resulted from new, previously unknown tertiary-structure motifs—was explained; the binding sites for tRNA and extraribosomal factors at the ribosomal A, P and E sites came into focus, subtle changes in rRNA structure were found to be involved in codon-anticodon interaction, peptidyl transfer and translocation. Perhaps most intriguing, early images of the peptidyl transferase center indicated that it was virtually devoid of proteins. The ribosome was proclaimed a ribozyme!

Yet there persisted evidence indicating vital roles for ribosomal proteins in protein synthesis: mutations in 30S subunit protein S12, for instance, showed its strong influence on accurate codon-anticodon interaction, while cross-linking experiments from my lab consistently showed that there was at least one protein, L27, present at the peptidyl transferase center of the 50S subunit. Our cross-linking strategy involved the substitution of the 3' adenosine of tRNA with a highly photoreactive nucleotide which, nonetheless, did not adversely affect aminoacylation, interaction with the ribosome, or peptide bond formation. When bound to either the A site or the P site, this tRNA derivative consistently formed cross-links to protein L27 as well as to nucleotides of the 23S rRNA known to be associated with peptidyl transferase center from other studies. Furthermore, because the cross-links resulted from a photoreactive nucleotide, they were essentially “zero-length,” that is, the 3' end of the tRNA was within very close proximity to L27, at a distance no greater than a single chemical bond.

The notion that L27 played a role at the peptidyl transferase center gained little traction at first because this protein was poorly resolved in early crystal structures of the bacterial 50S subunit and couldn't be precisely located. Moreover, it

was believed that peptidyl transferase activity could be fully accounted for by the involvement of specific nucleotides in the 23S rRNA. With improvements in crystal growth and analysis, however, Ramakrishnan's group demonstrated that in 70S ribosomes from *Thermus thermophilus* with A and P sites occupied by modified aminoacyl-tRNAs, several nucleotides at the 3' end of both tRNAs are within H-bonding range of specific amino acids within the N-terminal sequence of L27. Protein L27 is therefore capable of participation in peptidyl transfer by helping to orient and stabilize the “business ends” of the A- and P-site tRNAs, a key requirement for efficient catalysis. Potential interactions were also noted between L16 and the tRNA backbone at the A site. The ribosome is thus *almost* a ribozyme.

As the ribosome has shown us, the exceptional functional and structural properties of RNA are often linked synergistically to proteins that have evolved to stabilize and enhance their performance. Their cooperation is made possible by the many exposed functional groups that can interact in myriad ways via specific hydrogen bonds and electrostatic interactions. Our understanding of the mutuality between nucleic acids and proteins, ubiquitous in the world of gene expression, has been elucidated and refined over the past 20 years. In the world of RNA, it is manifested in numerous settings such as telomerase, transcriptional regulation, the processes by which RNA is modified and spliced, and in the many interactions of proteins with tRNA and the ribosome that underlie translation. Learning how the multitude of interactions between RNA and proteins have co-evolved will surely help us understand how the basic principles of protein-RNA recognition have led to the amazing complexity of gene expression as it exists today.

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