The evolution of RNase P

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About the time we were finishing our graduate and postdoctoral work, study of RNA biochemistry was electrified by the discovery of RNA catalysts and the proposal of the RNA World hypothesis. The key initial observations were Tom Cech's discovery of the *Tetrahymena* rRNA self-splicing intron and the joint discovery by the labs of Norm Pace and Sid Altman that bacterial RNase P, the endonuclease that cleaves pre-tRNAs to give mature 5' ends, was a *trans*-acting ribozyme. It soon became clear that the catalytic RNase P RNA subunit was widespread in Bacteria, largely from the work of Pace and colleagues. The excitement in the field was palpable over the notion that RNA (or something like it) was the primordial molecule, and many researchers immediately gravitated to the question of how much RNA catalysis survived in the modern world.

The enthusiasm for working out the mechanism of this ribozyme attracted contributions from many additional researchers, including Michael Harris, Tao Pan, Leif Kirsebom, Roland Hartmann, and Carol Fierke, among others. Characterization of the ribozyme activity of RNase P showed that the RNA component contained all of the determinants essential for binding and cleaving pre-tRNA. The activity requires magnesium ions and is activated by monovalent cations. Mutagenesis and phosphorothioate substitution experiments suggested metal binding sites and pinpointed the active site as in or near helix P4. Purification of the bacterial RNase P demonstrated that the *in vivo* enzyme was composed of a large RNA and a small protein component. The protein component facilitates cleavage under physiological conditions, enhancing the activity at low magnesium ion concentrations. Although the protein plays a role in stabilizing the structure of the RNA component, the Fierke lab demonstrated that the protein also directly interacts with the pre-tRNA leader and plays a role in pre-tRNA recognition. Furthermore, the protein component is important for facilitating an important biological function of RNase P, the cleavage of non-tRNA substrates. Kinetic studies of RNase P demonstrated properties of a highly evolved "perfect" enzyme where substrate association and product dissociation are rate-limiting for

steady state turnover. The laboratories of Norm Pace and Alfonso Mondragon made a big step forward in understanding RNase P by solving structures of the RNA, RNA-protein, and RNA-protein-tRNA complexes of bacterial RNase P that illuminated key features of this enzyme. These beautiful structures visualized the formation of coaxially stacked helical domains that recognize pre-tRNA based mainly on conserved structural features and confirmed the location of the active site in helix P4. These structures have enabled detailed studies of the catalytic mechanism, metal binding, and substrate recognition using single atom mutagenesis and other methods such that the understanding of the structure and function of this enzyme rivals that of many protein nucleases.

Work on the eukaryotic enzymes lagged behind the bacterial RNase P for a variety of reasons. Although the enzymatic activity was clearly present in crude cellular extracts, the enzyme was difficult to isolate to purity due to both low abundance and instability. Eventual isolation and characterization of the nuclear RNase P activity from yeast and human cells by the Engelke and Altman labs revealed several surprising aspects. First, although the large, catalytic RNA subunit of the enzyme was strikingly similar to the bacterial and archaeal subunits, especially in the highly conserved sequence "Critical Regions," there was twenty times as much protein content in the form of 9 or 10 required protein subunits. Subsequent mutagenesis of the yeast enzyme confirmed that the RNA was an essential catalytic subunit, even though the RNA alone was not a ribozyme (a tiny amount of activity was later coaxed from the human RNA subunit by Kirsebom's group). The immediate question became what was the function of all those proteins—surely not just to stabilize the RNA subunit or position it correctly in the nucleus?

One reason for the explosion in the number of protein subunits was suggested by another surprising aspect. At least 8 of the 9 RNase P proteins were also present in RNase MRP, an enzyme originally named for a role in mitochondrial RNA processing of replication primers, but later shown to be primarily localized to the nucleolus and involved in ribosomal RNA processing. RNase MRP was characterized primarily

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by the Craft, Clayton, Tollervey, Lindahl, Séraphin, van Venrooij, and Schmitt labs, confirming that the yeast and human P and MRP enzymes were similar to each other, sharing most protein subunits. In both yeast and mammals, however, the RNA subunit is similar, but significantly different in sequence. This suggested that RNase P had actually split into two enzymes in the nucleus (and nucleolus), classical RNase P to continue cutting tRNAs, and RNase MRP to cut pre-ribosomal RNAs (specifically in internal transcribed spacer 1): a beautiful example of “waste not, want not” in evolving an ancient RNA enzyme to multitask.

But there were still all of those protein subunits to account for. Was it possible that they were adapters so that the catalytic RNA sites of the enzymes could serve as endonucleases for more substrates? The short answer is yes. Mark Schmitt’s lab showed that RNase MRP was not only nucleolar and involved in pre-rRNA maturation, but also found in modest amounts in the cytoplasm, where it was involved in selective turnover of mRNAs, probably at the P bodies. This was followed by demonstrations in both yeast and human by the Engelke and Altman labs that RNase P could also cleave non-tRNA substrates, and in the case of yeast there was compelling evidence that the nuclear enzyme participated in turnover of long non-coding RNAs, including lncRNAs. Since the Engelke, Krasilnikov, and Schmitt labs showed that the purified RNase P holoenzymes had little or no sequence or structural specificity for their RNA substrates, it suggested that the protein subunits function as adapters—possibly guiding substrate selection by interaction with RNP proteins, as well as directly binding the other RNA substrates. This is strikingly reminiscent of the case with the eukaryotic transcription machinery, where there is proliferation of subunits on a catalytic core that is very similar between the simpler bacterial enzymes and the multiple nuclear enzymes. Although the functions of all of those extra subunits are still not resolved after 40 years, many of them are clearly involved in regulatory interactions with other protein complexes.

The characterization of the archaeal RNase Ps by the labs of Pace, Brown, Daniels, Kimura, Gopalan, and others revealed that archaea had split into both enzymes with a “simple” subunit composition akin to bacteria and more complex 4–5 subunit enzymes that more closely resembled the nuclear enzymes. But it was the question of how organelles (mitochondria and chloroplasts) dealt with tRNA 5′ end processing that led to considerable controversy. It was initially thought that the story would be analogous to bacteria, archaea, and nuclei, since Nancy Martin’s lab characterized the mitochondrial RNase P RNA from a number of fungi, and found that it had a protein subunit entirely distinct

from either the bacterial or nuclear enzymes. This turned out to be only an isolated biological branch, however, and the truth has turned into an interesting and possibly illustrative tale of convergent evolution, replacing what was probably an ancient ribozyme function. In the early 1990s Peter Gegenheimer’s lab had compelling evidence that there was an RNase P activity in spinach (chloroplasts) that had the physical properties and nuclease resistance of pure protein. Because at that time the chloroplast enzyme was not purified to homogeneity and cloned to confirm activity, there was room to question whether some combination of coincidences was only making this appear as a protein-only RNase P, and that the physiologically relevant enzyme was still a ribonucleoprotein. It is one of those ironies encountered in science that some of those investigators who were startled by the discovery of ribozymes, were resistant to the idea that there could be an RNase P without an RNA subunit. Similar skepticism was encountered by evidence of a protein-only RNase P in human mitochondria, reported first by Rossmannith and Karwan in the late 1990s, for similar reasons. It was not until Rossmannith carried out a tour de force experiment in 2008 to purify, clone, recombinantly express, and reconstitute the RNase P activity from human mitochondria that a protein-only RNase P was definitively demonstrated. The human enzyme requires three subunits for efficient pre-tRNA maturation, a nuclease from the PIN (PiT N-terminal) domain-like fold super-family, a tRNA methyltransferase, and a protein binding partner that has promiscuous alcohol dehydrogenase activity. Some eukaryotic species, such as *Arabidopsis thaliana*, seem to have given up the ribozyme form of RNase P entirely, being devoid of an RNA-based RNase P and instead utilizing a protein-only RNase P (PRORP) in all compartments. In *A. thaliana* three PRORPs are encoded within the nuclear genome localizing to the nucleus, the mitochondria, and chloroplasts. The structure of *A. thaliana* PRORP1, determined by the Fierke and Koutmos labs, visualized 3 domains: a metallonuclease domain, a central structural Zn-binding domain, and a pentatricopeptide repeat domain involved in pre-tRNA binding. The crystal structure elucidated the binding site of two manganese ions and activity assays demonstrated the catalytic importance of these metal ions. This enzyme is currently proposed to use a classical 2-metal ion mechanism, although additional studies are needed. The catalytic activity of the protein-only RNase P is not diffusion-limited and is therefore less efficient than the RNA-dependent enzyme, suggesting that enhanced catalysis was not the driving force for evolution of PRORP. The evolutionary forces that led to the development of a protein-only enzyme remain to be elucidated.

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