Recruiting more proteins to the RNA world

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Science, 362 (6415), • DOI: 10.1126/science.aav4743

BIOCHEMISTRY

Recruiting more proteins to the RNA world

A primordial but still essential ribozyme co-opts proteins as it evolves

By **William G. Scott**[1] *and* **Kiyoshi Nagai**[2]

Ribonuclease P (RNase P) recognizes precursor transfer RNA (pre-tRNA) and processes it to generate mature tRNAs that are used for assembling proteins. Unlike almost all other enzymes, RNase P is a ribozyme, an enzyme with an active site that is composed of RNA, and it is present in every living organism. RNase P is among the most ancient of enzymes, a living molecular fossil from an "RNA world" in which life is thought to have originated. On page 657 of this issue, Lan *et al.* (*1*) present structures of the yeast RNase P enzyme by itself and bound to its pre-tRNA substrate. Additionally, the structure of the human form, by itself and bound to its tRNA product, is reported by Wu *et al.* (*2*). These reveal the detailed mechanism by which RNase P hydrolyzes pre-tRNA to produce the required 5′-phosphorylated tRNA of exactly the correct length. These structures unambiguously reveal how an assortment of proteins conspire to form a measuring device that ensures that the pre-tRNA substrate is correctly processed by the catalytic RNA subunit of this universal and essential enzyme.

RNase P was discovered by Robertson, Altman, and Smith in 1972 (*3*), who described an enzyme in the bacterium *Escherichia coli* that precisely removes the 5′ end of pre-tRNAs to produce mature tRNA products,

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Life is proposed to have begun with an RNA or RNA-like molecule.

the adaptor molecules that are required for translating the genetic code into proteins. A decade later, Altman, Pace, and colleagues published a remarkable discovery: The RNA subunit of bacterial RNase P is the active catalytic component (*4*). Before this discovery, and the nearly simultaneous discovery by Zaug and Cech of self-splicing RNA (*5*, *6*), it was assumed that only proteins could be enzymes. Altman and Cech soon shared the Nobel Prize, and added renewed traction to the "RNA world hypothesis" (*7*), which proposes that life began with RNA or an RNA-like molecule that can carry both genetic information and catalytic activity.

Although the RNA subunit in bacterial RNase P is catalytic, the enzyme also has a single protein subunit that appears to play an ancillary role in substrate recognition. More complex versions of RNase P are found in *Archaea* and *Eukarya*. Although they all possess similar RNA subunits, the archaeal and eukaryotic versions also contain multiple proteins. Are these more complicated RNase P enzymes also ribozymes? Or has evolution replaced the catalytic component with more conventional protein active sites? The new structures of yeast and human RNase P finally answer this question and in so doing provide a fascinating glimpse of the evolutionary transition between the RNA and protein worlds.

In addition to the RNA subunit, yeast RNase P has 10 protein subunits. The yeast RNase P RNA has a more elaborate structure compared with its more compact bacterial counterpart, but it shares a similar central region (*8*, *9*). In the yeast enzyme, the proteins form a "hook" that wraps around the RNA subunit, stabilizing the entire structure. The resulting protein-RNA complex forms a precise measuring device that targets pre-tRNAs for hydrolysis by the enzyme's active site, which is composed entirely of RNA (see the figure). The new structures show that yeast RNase P, despite being adorned with proteins, is indeed still a ribozyme.

Similar to bacterial RNase P, the active site of the yeast enzyme reveals two Mg^{2+} cations coordinated by adjacent nonbridging phosphates within the backbone of the RNA subunit, as well as the phosphate at the cleavage site of the pre-tRNA. One of the Mg^{2+} ions is also coordinated by a conserved nucleotide base in the RNA, as previously observed in the bacterial enzyme (*8*, *9*)—apparently the only nucleotide base that participates directly in catalysis. Many of the other known ribozymes also have active sites with two Mg^{2+} cations, which is consistent with an early but insightful prediction (*10*); they are observed in every ribozyme involved in RNA splicing, including the spliceosome (*5*, *6*, *11*–*14*). In the case of RNase P, one of the Mg^{2+} ions activates a water molecule to hydrolyze (break) the phosphodiester backbone of the pre-tRNA substrate. This geometrical arrangement of the reactants explains not only how the phosphate comes to reside on the 5′ end of the product tRNA as required but also how a more typical RNA degradation reaction, which could result from phosphodiester isomerization, is prevented. Lan *et al.* also provide a thorough and compelling set of computational reaction simulation results, demonstrating the plausibility of their proposed tRNA cleavage mechanism.

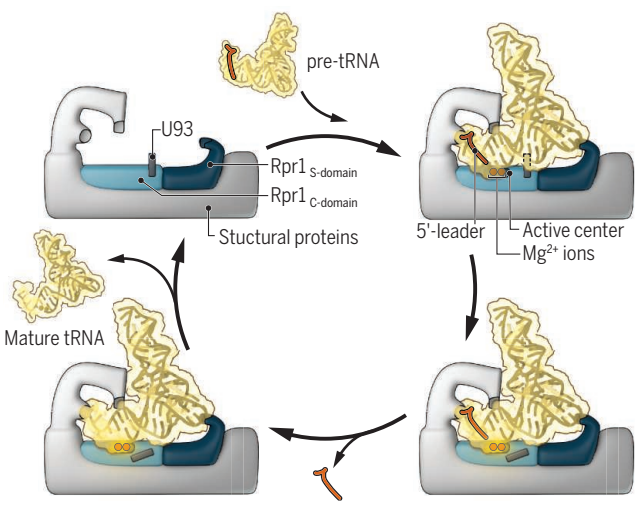
Like the yeast enzyme, human RNase P has one RNA (H1) and 10 homologous proteins. Therefore, the architecture of the holoenzyme is similar to that of yeast RNase P, and the mechanism of tRNA recognition is conserved. However, in the human structure, the 5′ end of the bound tRNA product is disordered, and the structure does not reveal bound catalytic metal cations (*2*).

Much of our prior knowledge of the recognition mechanism of RNase P was based on the crystal structure of bacterial RNase P, in complex with the mature tRNA reaction product (*9*). The single protein subunit in the bacterial RNase P guides binding of the 5′ leader sequence of pre-tRNA. The multiple protein components in yeast and human RNase P stabilize the catalytic RNA subunit and similarly guide pre-tRNA binding.

Many intriguing questions about the origin, evolution, and function of RNase P sharpen in focus with these new structures. Why has eukaryotic RNase P recruited many more noncatalytic protein subunits, given that bacterial RNase P, with a single protein subunit, is optimized for tRNA processing? Do these proteins extend the functionality of RNase P in processing other structural RNAs in eukaryotic cells? A multitude of other, entirely protein-based RNase enzymes exist, even including some forms of RNase P (*15*). Despite this, with one intriguing exception to the rule (*16*), the ribozyme version of RNase P is found in every cell of every organism, implying not only that this ribozyme is indeed a living fossil that dates back to the original life forms, but that the catalytic RNA subunit has been rigorously conserved during several billion years of evolution instead of being replaced with recruited proteins. What is it about this RNA that is so indispensable? The same type of hydrolysis reaction can be catalyzed by a variety of proteins that possess an RNase III catalytic domain. The new structures provide us with many intriguing hints. ■

Eukaryotic RNase P tRNA processing

The yeast RNase P consists of ten structural proteins and one RNA subunit (Rpr1). The Rpr1 specificity (S) domain and the protein subunits form a measuring device that positions the pre-tRNA at the active center. The Rpr1 catalytic (C) domain contains a conserved nucleotide (U93) that coordinates one of two Mg^{2+} ions, which are involved in catalyzing pre-tRNA cleavage.



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