New Horizons for RNA Catalysis

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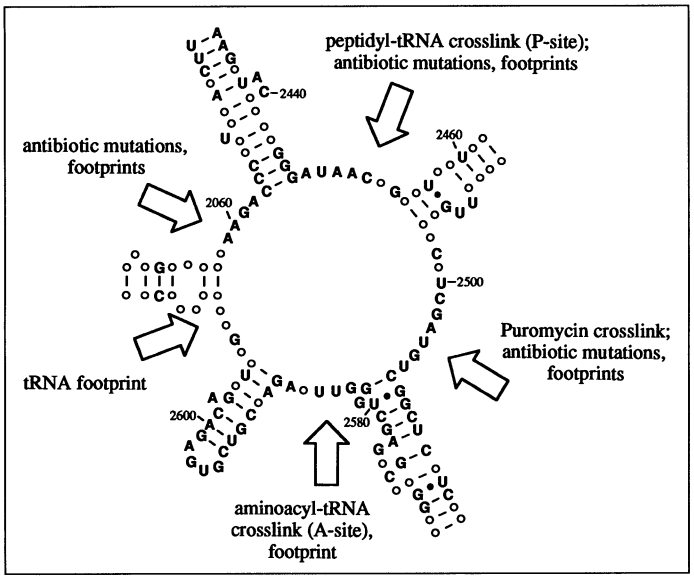
Two noteworthy reports in the annals of RNA-mediated catalysis appear in this issue of *Science*. One, by Noller *et al.* (*1*), provides strong evidence that a central step in protein biosynthesis—formation of a peptide bond—is catalyzed by an RNA moiety of the ribosome. The second, by Piccirilli *et al.* (*2*), corroborates the RNA chemistry suggested in Noller's results by showing that a previously studied ribozyme (an enzyme composed of RNA) can hydrolyze an aminoacyl ester. These findings expand the known repertoire of ribozyme chemistry beyond the making and breaking of phosphodiester bonds.

The ribosome has received intense scrutiny over the past 35 years, but only recently has attention been drawn to the ribosomal RNA (rRNA) as potentially responsible for the catalytic mechanism of protein synthesis (*3*). Earlier, conventional wisdom held that the protein components of the ribosome must be responsible for catalytic activity because only proteins were known to have catalytic potential. Although RNA comprises about 60 percent of the ribosomal mass, it was viewed as an inert scaffold on which to organize the proteins and to base-pair with other RNA's involved in the process.

Several factors nudged the rRNA to center stage. One was that it proved difficult to assign functions to particular ribosomal proteins, and indeed many could be deleted from *Escherichia coli* without lethal effects. Another factor was that most mutations resulting in resistance to inhibitors of protein synthesis seemed to be associated with the rRNA, not the protein genes. Moreover, the structure of rRNA proved very much more highly conserved throughout evolution than that of the proteins (*4*), an indication to many workers that RNA *had* to be the seat of action in the ribosome. The problem with that idea was that RNA was thought incapable of catalyzing chemical reactions, a presumption that was shattered with the characterization of a catalytic RNA by Cech (*5*). Subsequently, thought on the mechanism of ribosome activity turned to its RNA elements. Until the work of Noller *et al.* (*1*), however, no specific chemical reaction had been associated with the rRNA.

Noller *et al.* chose for their analysis of rRNA function the peptidyl transferase reaction. In this reaction the ribosome transfers a growing peptide chain from peptidyl tRNA to an amino acid esterified with another tRNA, forming a peptide bond. There has long been reason to suspect that a small portion of the large subunit (23S-like) rRNA is involved in that process, based on interactions with antibiotics and cross-linking studies (*3*). Moreover, this "peptidyl transferase center" (Fig. 1) contains sequences that are among the most highly conserved in biology.



Secondary structure of peptidyl transferase center. Arrows, regions implicated in peptidyl transferase activity on the basis of mutation, crosslink, and footprint data (*1*); boldface, nucleotides conserved in 90 percent or more of all organisms; open circles, nucleotides less than 90 percent conserved; bars, base pairs; black dots, non–Watson-Crick base pairs. Numbering according to *E. coli* 23S rRNA. [Art and sequence comparisons by J. W. Brown and R. R. Gutell]

As an assay for peptide bond formation, Noller *et al.* used a mimic, the "fragment reaction," in which a model peptide (*N*-formylmethionine) associated with a fragment of tRNA is transferred by the ribosome to the amino group of puromycin, an aminoacyl tRNA analog. They discovered that bacterial ribosomes are appreciably active in catalyzing this reaction even after destruction of proteins by treatment with high concentrations of proteinase in the presence of a strong detergent and solvent extraction to remove residual peptides. Critically, the observed transfer of the amino acid to puromycin was sensitive to antibiotics that block peptidyl transferase and insensitive to antibiotics blocking other events in protein synthesis. Trace contamination of treated ribosomes with critical peptides cannot yet be ruled out rigorously, but the study offers strong evidence that the rRNA occupies a central, catalytic role in protein synthesis.

The report by Piccirilli *et al.*, from the Cech laboratory, beautifully complements the ribosome work with the demonstration that RNA can perform the chemistry required for the first step of the peptidyl transferase reaction, in which the peptide is released from the tRNA. Cech and colleagues exploited a ribozyme that does not normally carry out this reaction, the self-splicing intron from *Tetrahymena thermophila*. The intron normally catalyzes its own excision from another RNA by cleaving at the intron boundaries and religating the host RNA. Thus, the natural substrate of the intron is the RNA phosphodiester, as is the case with all other known ribozymes (*5*). Piccirilli *et al.* altered the intron so that it could bind the same amino acid–tRNA fragment used by Noller in the ribosome experiments, in a manner that should position the aminoacyl group in close proximity to the active site of the ribozyme. The intron indeed catalyzed hydrolysis of the ester, albeit slowly. Any catalysis at all is remarkable considering the different chemistries of the aminoacyl ester and the phosphodiester. The details of the mechanism of the RNA-catalyzed hydrolysis of the aminoacyl ester remain to be resolved and may differ from the mechanism used by protein esterases, where different functional groups are available.

These new findings fuel long-standing speculation that RNA played the central role in the origin of life (*6*). Only RNA is known to be capable of the chemistry required for its own replication and, now, in addition for making protein. Protein synthesis is complex, but conceivably all the required players are RNA: tRNA, mRNA, and rRNA, the catalyst. It may be possible to understand the mechanism of protein synthesis in the context of a rudimentary translation apparatus com-

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posed solely of RNA. The recognition of further catalytic versatility in RNA justifies consideration of its involvement in other early biochemistry. Recently, in vitro selection and amplification methods (*7*) have opened the way to the exploration of new RNA functions, without the constraints of biology. Over the coming years we may expect to see even further expansion of the catalytic capacity of RNA.

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On the Other Hand . . .

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Almost 25 years ago my colleague, Prof. Dagmar Ringe, was asked during her Ph.D. thesis defense to speculate on what would happen if a proteolytic enzyme (one that cuts the peptide strand of a protein) could be made from all D-amino acids instead of the naturally occurring L-enantiomers. She replied that the protein would probably fold properly and have full catalytic activity, but only against peptide substrates of the same chirality. Her examiners did not believe her (she passed anyway), but now, a quarter of a century later, the experiment has been done and that is exactly what happens. Stephen Kent and his associates at the Scripps Research Institute have achieved the total chemical synthesis of an all-D-amino acid enzyme, the acid protease from the human immunodeficiency virus (HIV). As reported in this issue of *Science* (*1*), the protein displays a chiral specificity for both substrates and inhibitors that is the opposite of that shown by the naturally occurring all-L-amino acid enzyme.

"Chiral" comes from the Greek word for "hand," and the term "handedness" is often used interchangeably to describe the same property. An object is chiral if it cannot be superimposed on its mirror image by simple rotations and translations. The quintessential example of two objects of opposite chirality are the left and right human hands (see figure). Two objects of opposite handedness can always interact with the same achiral object: for example, both the left and right hands can hold a baseball in the same way. But their interactions with another chiral object must be different, and in some cases only one of them may be able to interact at all. You cannot use a left-handed baseball glove on your right hand. (Another example: Two right hands can shake with each other, as can two left hands. The left and right hands can also clasp, but one must turn upside down to do so.) Molecules are chiral if they lack a center, plane, or axis of symmetry. If they have any one of these, they will be superimposable on their mirror images.

Chirality is fundamental in biology. The building blocks of proteins, the naturally occurring amino acids, are chiral molecules. And in all proteins from all living organisms studied thus far, all amino acids are of one particular handedness: the so-called L-configuration (the nomenclature of chirality is changing at present, and for amino acids R- and S- are slowly replacing the older D- and L-designations). Some lower organisms use D-amino acids in certain specialized molecules, like cell walls and antibiotics, but proteins are always composed entirely of L-residues. Such chiral purity has advantages that have long been appreciated: as the baseball analogy indicates, a chiral protein has more specific recognition of, and interaction with, chiral substrates. That is why it was reasonable to expect that a proteolytic enzyme will only recognize one enantiomeric peptide substrate: its active site is chiral and the relative position of the peptide bond to be cleaved will be different in substrates of opposite handedness. Invert the hand of the enzyme and you must also invert the hand of the substrate in order to make the same interactions and obtain catalysis. A further reason for chiral proteins is that secondary structural elements such as alpha helices and twisted beta sheets have a handedness of their own, which is predicated on a consistently handed set of amino acid residues. Thus, all L-amino acid polymers make right-handed alpha helices whereas homopolymers of D-amino acid residues form left-handed ones. The reason is steric: there are unacceptable side chain clashes in a right-handed helix containing D-amino acids. Finally, chiral purity is necessary for the protein synthesizing machinery. It is essential to be able to assume that the side chain will always be in the same relative position with respect to the carboxylate and amino portions of every amino acid.

Symmetry's handiwork. The left and right human hands cannot be superimposed by simple rotations and translations; they are mirror images of each other. [Woodcut by Albrecht Dürer (1471–1528), from the Bettmann Archive]

So it is easy to rationalize why we have a chiral world, but it is not so easy to explain how we got the one we have. Why L-amino acids? Was the choice between L- and D- a random one, or does it offer some clues as to the early biochemistry of life? Prebiotic experiments in which, for example, electrical discharges are passed through mixtures designed to mimic the chemical composition of the primordial atmosphere, produce racemic products, that is, equal amounts of both D- and L-amino acids. It has been suggested that the choice of L- reflects an asymmetry in the environment in which the first proteins were formed: mineral deposits, or clays perhaps. Yet most such substances are themselves racemic, so the chance of encountering something that would favor L-amino acids is presumably random. It has also been suggested that the earliest life forms were imported onto Earth from other planets, so that the chiral preference we see is simply a reflection of what existed elsewhere. This panspermia hypothesis, of course, merely transfers the problem of choice of hand—as it does the problem of the origin of life—outside of our purview and so is really no help at all. Another, more productive, rationale is that beta decay is intrinsically asymmetric and will preferentially destroy D-amino acids (*2*). The discrimination, however, is only a few percent and probably not a sufficient explanation for the observed preference. We are left then with the view that the choice was probably random. For that to be possible, it would be helpful to

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