PERSPECTIVES: STRUCTURAL BIOLOGY

The Ribosome Is a Ribozyme

Thomas R. Cech

The amino acids we obtain by digestion of steak, salmon, or a lettuce salad are loaded onto transfer RNAs (tRNAs) and rebuilt into proteins in the ribosome, the cell's macromolecular protein-synthesis factory. The bacterial ribosome is composed of three RNA molecules and more than 50 proteins. Its key components are so highly conserved among all of Earth's species that a similar entity must have fueled protein synthesis in the common ancestor of all extant life. Although the chemical reaction catalyzed by the ribosome is simple—the joining of amino acids through amide (peptide) linkages—it performs the remarkable task of choosing the amino acids to be added to the growing polypeptide chain by reading successive messenger RNA (mRNA) codons. On page 905 of this issue, Steitz, Moore, and colleagues (*1*) now provide the first atomic-resolution view of the larger of the two subunits of the ribosome. From this structure they deduce on page 920 that RNA components of the large subunit accomplish the key peptidyl transferase reaction (*2*). Thus, ribosomal RNA (rRNA) does not exist as a framework to organize catalytic proteins. Instead, the proteins are the structural units and they help to organize key ribozyme (catalytic RNA) elements, an idea long championed by Harry Noller, Carl Woese, and others.

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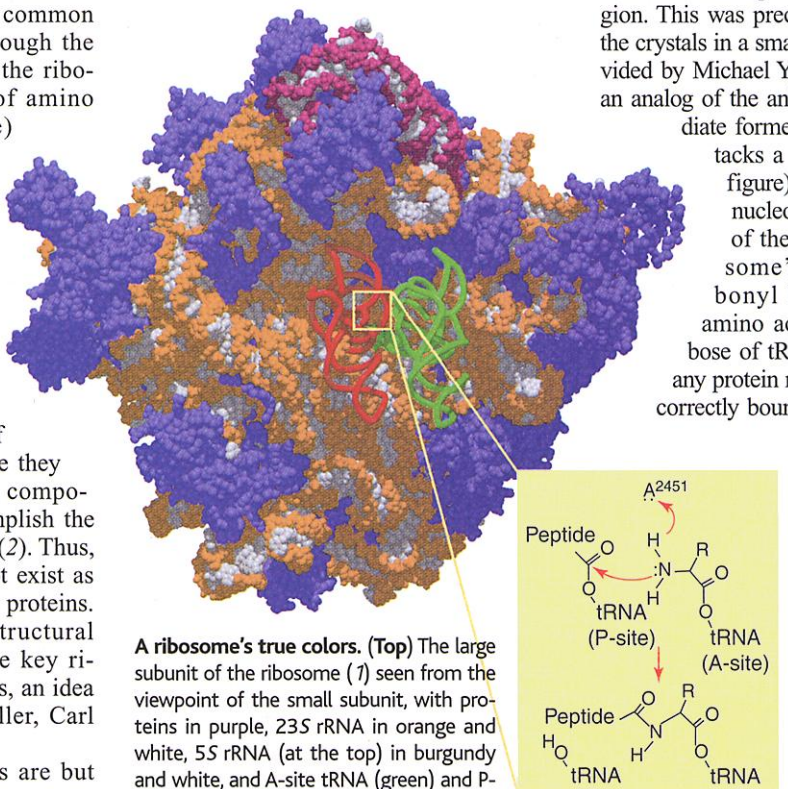
These landmark publications are but the latest chapter in a progression of ribosome structural studies that have spanned four decades. Early electron micrographs of ribosomes in action led to immunoelectron microscopy and ultimately to cryo-electron microscopy images of about 20 Å resolution. Proteins were also located within the ribosome by neutron scattering. However, to achieve atomic resolution, x-ray crystallography is required, a daunting task given the huge size (2.6×10^6 daltons) and asymmetry of the ribosome. The pioneering crystallization of ribosomes from the bacterium *Haloarcula marismortui* in the 1980s by Ada Yonath and H. G. Wittmann provided the first rays of hope, but it is only in the past few years that crystal structures have been determined for the large subunit (5 Å resolution) (*3*), the small subunit (5.5 Å resolution) (*4*), and the whole ribosome complexed with tRNAs (7.8 Å resolution) (*5*).

Now, at 2.4 Å, almost the entire chain of the 23*S* rRNA and its tiny 5*S* rRNA partner, totaling 3043 nucleotides, have been fitted into the electron density map of the *H. marismortui* large ribosomal subunit (*1*). The RNA secondary structure (intramolecular base-pairing pattern) of the large-subunit rRNA had been determined previously (*6*), and is present as predicted in the x-ray structure. In addition, a large number of unpredicted RNA tertiary structure interactions are now seen. Overall, the RNA forms a huge single mass of tightly packed helices, not six discrete domains connected by floppy linkers as a naïve observer might predict from looking at the secondary structure diagram.



A ribosome's true colors. (Top) The large subunit of the ribosome (*1*) seen from the viewpoint of the small subunit, with proteins in purple, 23*S* rRNA in orange and white, 5*S* rRNA (at the top) in burgundy and white, and A-site tRNA (green) and P-site tRNA (red) docked according to (*5*). **(Bottom)** The peptidyl transfer mechanism catalyzed by RNA (*2*). The general base (adenine 2451 in *Escherichia coli* 23*S* rRNA) is rendered unusually basic by its environment within the folded structure; it could abstract the proton at any of several steps, one of which is shown here.

Where, then, are all of the proteins, and what is their function? The globular domains of 26 proteins are found largely on the exterior of the subunit (see the figure). Twelve of these proteins have unusual snake-like extensions, devoid of tertiary structure and in some cases even secondary structure, and an additional protein is entirely extended; their shapes are molded by their interactions with the RNA. From these pictures, and from what is known about protein cofactors that facilitate the action of some other ribozymes, it is likely that these ribosomal proteins buttress, stabilize, and orient the otherwise floppy RNA into a specific, active structure.

The part of the subunit's surface that is most devoid of protein is the active-site region. This was precisely located by soaking the crystals in a small-molecule inhibitor provided by Michael Yarus (*7*). This inhibitor is an analog of the anionic tetrahedral intermediate formed when a nucleophile attacks a planar carbonyl (see the figure). (In protein synthesis, the nucleophile is the amino group of the amino acid in the ribosome's A-site, and the carbonyl belongs to the P-site amino acid esterified to the 3′-ribose of tRNA.) It is the absence of any protein moiety within 18 Å of the correctly bound inhibitor in their structure, coupled with earlier work that defined this conserved part of the large-subunit rRNA as the "peptide transferase center," that led the authors to conclude that RNA (and not protein) must be responsible for catalysis. The ribosome is a ribozyme, admittedly one dependent on structural support from protein components—substantially deproteinized large subunits still carry out peptidyl transfer, although complete deproteinization destroys this reactivity (*8*).

The authors propose a detailed mechanism for catalysis (*2*) that will undoubtedly be the subject of much analysis and experimental testing. One key feature of the mechanism is a particular adenine base (conserved at this position in thousands of sequenced rRNAs) that acts as a general acid-base catalyst, deprotonating the nucleophilic amine (see the figure) and protonating the 3′-oxygen of the ribose reaction

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product. The ability of RNA to provide general acid-base catalysis was discovered only last year (*9,10*) in studies involving the hepatitis delta virus ribozyme (*11*).

Efficient general acid-base catalysis requires that the acid-base have a pK_a around pH 7.0, whereas the adenine base titrates at or below pH 3.5. However, it is already known that certain RNA structures can perturb the pK_a of adenine toward a neutral pH (*12*). In addition, as Muth *et al.* (*13*) report on page 947 of this issue, experimental analysis of the nucleotides within the peptidyl transferase center demonstrates that the adenine implicated by the crystal structure has an unusual pK_a of 7.6. Remarkably, two RNAs—identified by in vitro evolution for their ability to catalyze peptidyl transfer (*14*) or to bind the analog of the reaction intermediate (*15*)—have adenines in a local sequence and secondary structure similar to that of the critical adenine in the ribosome. So, this pair of RNAs may recapitulate the key feature of the rRNA reaction mechanism.

Of course, general acid-base catalysis can easily be provided in the active site of a protein enzyme, which leads to the question: Why does nature use RNA catalysis to achieve protein synthesis? One argument is evolutionary. If, indeed, there was an early RNA world where RNA provided both genetic information and catalytic function, then the earliest protein synthesis would have had to be catalyzed by RNA. Later, the RNA-only ribosome/ribozyme may have been embellished with additional proteins; yet, its heart of RNA functioned sufficiently well that it was never replaced by a protein catalyst. But there are persuasive chemical arguments as well. The substrates of the ribosome are RNAs—aminoacylated tRNAs and an mRNA—and RNA is particularly well suited for specific recognition of other RNAs through formation of base pairs, base triples, and other interactions. Furthermore, RNA is well suited to perform very large-scale conformational changes, and such movements are required for protein synthesis.

These most recent contributions of Steitz, Moore, and colleagues provide a milestone, but not the finish line. This one structure contains more RNA-RNA and RNA-protein interactions than all previous atomic-level structures combined, so ribophiles can look forward to years of additional analysis. The whole ribosome needs to be brought to this same atomic level of resolution, and the proposed reaction mechanism deserves critical testing. Finally, the molecular basis of the mRNA translocation step that must occur after each peptidyl transfer event remains obscure. Thus, although the current crystal structure provides one beautiful frame, we still look forward to seeing the entire movie.

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PERSPECTIVES: MATERIALS PROCESSING

The Power of Direct Writing

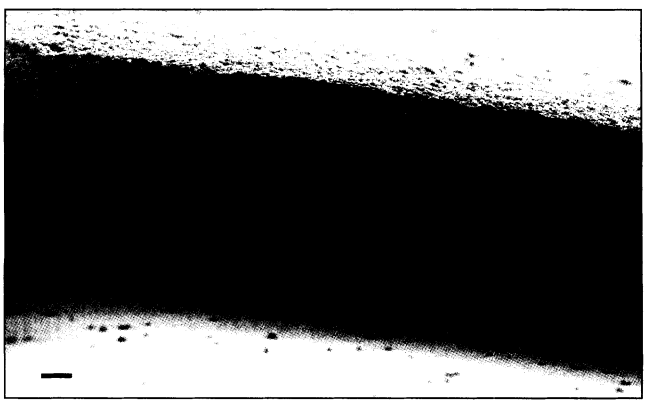
Douglas B. Chrisey

Direct-write technologies are of increasing importance in materials processing, enabling, for example, the simplification of printed circuit board manufacture at reduced costs (*1*). In a direct-write approach, structures are built directly without the use of masks, allowing rapid prototyping. As materials and processing challenges are being met with increasing success, direct-write techniques move toward a wide range of applications. Passive electronic components and interconnects have been made by direct-write techniques using a variety of materials. In a parallel development, direct writing of biomaterials is used for tissue engineering and array-based biosensors.

To optimize different direct-write techniques, electronic materials and approaches must be tailored for each processing method, transfer method, and required electronic or other device performance. Many different approaches exist to direct-write or transfer patterned materials, and each technique has its own merits and shortcomings. The techniques include plasma spray, laser particle guidance, matrix-assisted pulsed-laser evaporation (MAPLE), laser chemical vapor deposition (CVD), micropen, ink jet, e-beam, focused ion beam, and several novel liquid or droplet microdispensing approaches (*2–9*).

One theme common to all techniques is their dependence on high-quality starting materials, typically with specially tailored chemistries and/or rheological properties (such as viscosity, density, and surface tension). The starting materials, sometimes termed "pastes" or "inks," may consist of combinations of powders, nanopowders, flakes, surface coatings, organic precursors, binders, vehicles, solvents, dispersants, and surfactants. These materials have applications as conductors, resistors, and dielectrics and are being developed specifically for low-temperature deposition (<300° to 400°C). They will allow fabrication of passive electronic components and radio frequency devices with the performance of conventional thick film materials, but on low-temperature flexible substrates, such as plastics, paper, and fabrics. The desired final electronic materials may be silver, gold, palladium, and copper conductors or alloys; polymer thick film and ruthenium oxide–based resistors; and metal titanate-based dielectrics.

Problems arise, however, because the fabrication of high-quality crystalline materials—required for high electronic performance of the final material—is nearly impossible at



Tight packing. This scanning electron micrograph of a fracture cross section demonstrates the extremely uniform and optimized packing of $BaTiO_3$ nanopowders. The individual powder particles have assembled to produce a dense dielectric layer.

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