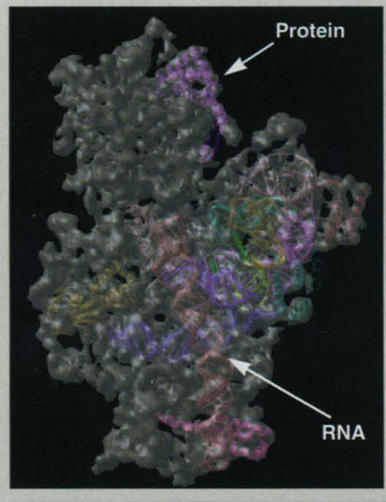
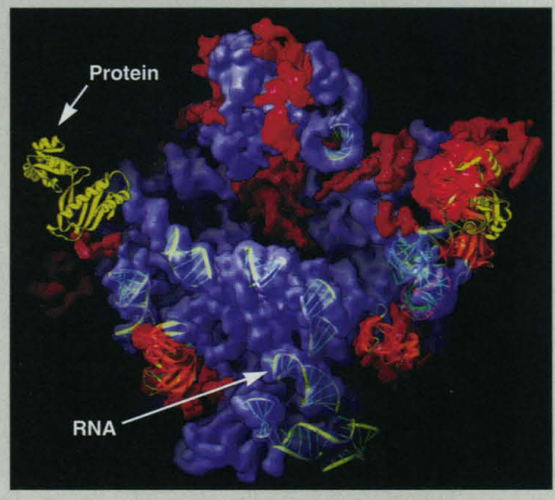
Ribosome Finally Begins to Yield Its Complete Structure

Crystallographers have long regarded the ribosome, the cell's protein factory, as the Mount Everest of their field. In fact, many have considered it insurmountable. A conglomerate of some 54 different proteins and three RNAs, it lacks the symmetry and repetitions that have eased the way to solving the structures of bigger entities, such as viruses. Now, two research teams have succeeded in setting up base camp.

Crystallographer Thomas Steitz and biophysical chemist Peter Moore of Yale University and their colleagues report in the 26 August issue of *Nature* that they have worked out the structure of the larger of the two distinct subunits that form a complete ribosome. And in an accompanying paper, an independent team led by Venki Ramakrishnan, a biochemist who has just moved from the University of Utah, Salt Lake City, to the Medical Research Council Laboratory of Molecular Biology in Cambridge, U.K., has determined the structure of the smaller subunit. Both teams attained a resolution of about 5 angstroms. Although still too fuzzy for atomic detail, the images show the overall arrangement of the proteins with respect to the RNAs—enough detail that researchers consider this a milestone for ribosome studies.

For years, ribosome experts have had to make do with indirect approaches to studying the ribosome's structure, such as mutating the various components to see how the changes affect protein synthesis. With the new structures, says Joachim Frank, a physicist at the New York State Department of Health Wadsworth Center in Albany, "we can now begin to put all the bits and pieces together." These structures and higher resolution ones expected within the next year or two represent "a first step in a whole new direction," agrees Albert Dahlberg, a molecular biologist at Brown University in Providence, Rhode Island, that will reveal just how these proteins and RNAs interact as they piece together a protein's amino acids.





Sneak peek. Newly derived electron density maps of the ribosome's 30S (*top*) and 50S (*bottom*) subunits reveal where the proteins and RNAs are relative to one another.

Ramakrishnan's team tackled the smaller ribosome subunit—designated the 30S subunit from the rate at which it sediments in the ultracentrifuge—from the bacterium *Thermus thermophilus*. It took 2 years of tinkering to get the material they needed—good crystals with heavy atoms inserted as landmarks for interpreting x-ray diffraction data. From the 140 crystals they screened at the National Synchrotron Light Source at Brookhaven National Laboratory in Upton, New York, they were able to use six to build maps of the electron density of the subunit. The detail was sufficient to reveal certain key features, such as where the RNA had looped back on itself to form a double helix and where the amino acids of the proteins formed distinctive spirals called α helices, says Brookhaven's Malcolm Capel, who helped both teams with their diffraction studies.

Aided by a neutron-scattering map that showed the approximate locations of all the proteins in the subunit, plus the structures of seven that had already been individually determined by Ramakrishnan and Stephen W. White of St. Jude Children's Research Hospital in Memphis, Tennessee, and others, Ramakrishnan's team also managed to pinpoint the positions of eight of the 21 proteins. In addition, with help from biochemical data previously obtained by others indicating which proteins are in contact with the RNA, Brian Wimberly in Ramakrishnan's lab traced the RNA's path in the center of the molecule.

Meanwhile, Moore and Steitz's team focused on the structure of the larger, 50S ribosomal subunit, which they obtained from a salt-loving microbe, *Haloarcula marismortui*. Some 20 years ago, biocrystallographer Ada Yonath of the Weizmann Institute of Science in Rehovot, Israel, had shown that it was possible to crystallize this subunit, but she and others have been unable to generate detailed x-ray diffraction data. Moore and Steitz found that part of the problem may be the fact that the giant molecule—containing 34 proteins and two RNAs—tends to make twinned crystals. Until one realizes twinning occurs, these look fine but ultimately give confusing results when studied using x-ray diffraction. Consequently, says Moore, they wound up rejecting the majority of the crystals they made. For this reason, and because of its size, "this was a bigger mountain to climb than was the 30S subunit," Steitz notes.

In many cases, the structures confirmed what researchers already thought. For example, Steitz and Moore's team could see a tunnel in the 50S subunit that others had observed in electron microscopy studies. But there were surprises as well. The structures indicate, for example, that protein-protein interactions may be more important than thought in making sure the right amino acid is picked during protein assembly. Previous research had shown that mutations in two particular 30S proteins cause mistakes in that selection, and researchers had assumed that the mutations affect the proteins' interaction with the ribosomal RNA. But the structure shows that the mutations actually affect the interface between these two proteins. "This is a hint that the proteins are actually doing something," says Ramakrishnan.

He and others expect many more surprises as they improve crystallization, diffraction, and analysis to achieve ever higher resolution. At least two other teams, Yonath's and that of Harry Noller of the University of California, Santa Cruz, expect to publish ribosome structures in the upcoming months, setting the stage for an intense race for the first high-resolution one. And, that, says Moore, is what makes these particular images so exciting: "Having gotten this far, it's now clear that we can go the rest of the way."

–ELIZABETH PENNISI

CREDITS: (TOP TO BOTTOM) RAMAKRISHNAN *ET AL.*; N. BAN, P. NISSEN, P. B. MOORE AND THOMAS A. STEITZ