

BUILDING A

Blueprint

OF THE CELL'S

PROTEIN FACTORY

JOACHIM FRANK

If the genome is the blueprint that defines an organism and directs every facet of its operation, then the ribosome is where the bare-knuckled work of carrying out those instructions takes place. A typical human cell contains millions of these protein-building factories, where the 20 different amino acids derived from foods are linked together—one at a time, accurately and rapidly—into the proteins needed for life.

“Without ribosomes, you’re dead,” says Thomas A. Steitz, an HHMI investigator at Yale University. “But despite their vital role, major questions have persisted about how ribosomes are built at the atomic level and about how they promote the synthesis of proteins. We’ve now answered a big part of that first question, and we’ve provided some of the first hard evidence that will help in understanding the second part.”

Steitz and a small group of talented researchers assembled a richly detailed, three-dimensional map of the structure of the ribosome that promises to clarify the fundamental process of protein synthesis. The implications go even further, however, offering new insight into how life may have evolved on Earth and suggesting a more focused approach to designing antibiotics.

“This work is a scientific tour de force,” says Robert Haselkorn, a molecular geneticist at The University of Chicago and president of Integrated Genomics, a biotechnology company based in Chicago. “Since the critical role that ribosomes play in cell metabolism was first identified half a century ago, scientists

have wanted to better understand the structure of these massive molecules and to learn exactly how they do their work as protein builders. Tom Steitz and his colleagues have really nailed this information down, and we now know with assurance the most intimate details of the ribosome.”

As with most scientific achievements, the work builds on a foundation established by the thousands of scientists who have pored over every aspect of ribosome function during the past 50 years. Through the years, research groups large and small have amassed an impressive amount of information about the ribosome, but no group has succeeded in creating an accurate three-dimensional map.

“We decided to take a different approach,” Steitz says. “Rather than assemble an army, we brought together just a few really good researchers to work very closely with each other. In this way, all of us knew everything about the project, so each person was prepared to contribute and critique ideas on every aspect of the problem. We met every Friday for lunch—no seminars, no slides, just open discussion of what we were doing. Looking back, I think that was important. We didn’t think about it when we began, but later someone observed that what we had assembled was really a guerilla force rather than an army. Whatever we had, it seemed to work.”

Steitz assembled a core team that included postdoctoral researchers Nenad Ban, Poul Nissen and Jeffrey Hansen, along

*The awesome size of the ribosome demanded a different approach, says Thomas A. Steitz.*

BY TOM BURROUGHS

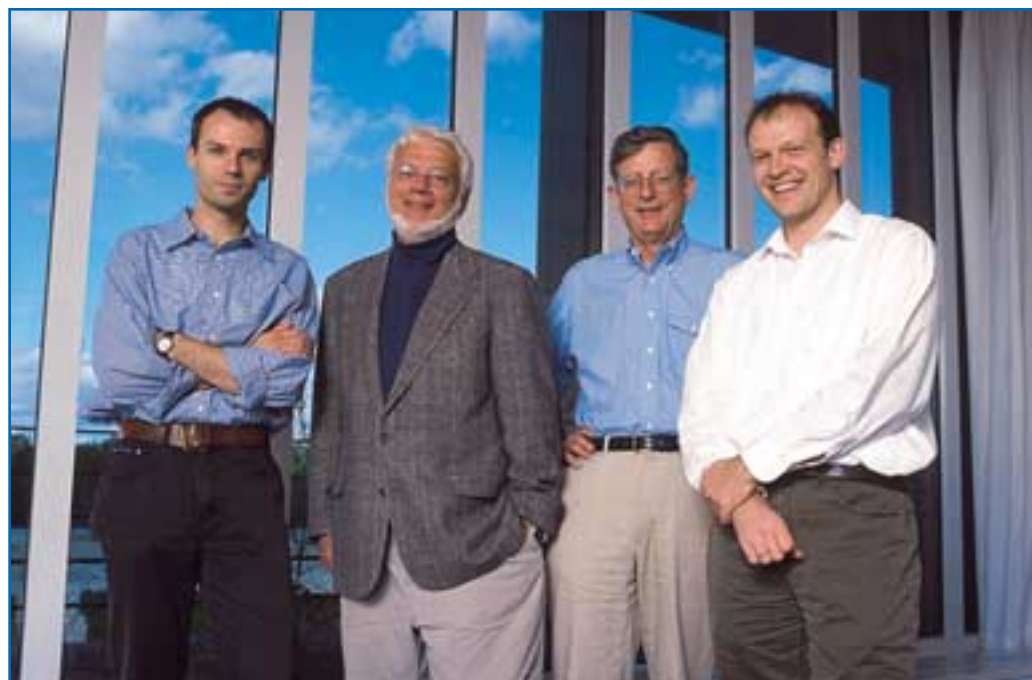
with Peter Moore, a professor of chemistry at Yale. “I had known Peter for a long time—we had been graduate students together—and for a decade or more he and I had been kicking around the idea of tackling this project,” Steitz says. “When you’re considering something that seems this hopeless, given how long the problem had resisted solution, it helps to have someone like Peter just down the hall.”

In Moore, Steitz had a person who knew ribosome behavior and, more important, knew how to grow ribosome crystals in such a way that the “frozen” ribosomes retained much of their structure. In Steitz, Moore had a seasoned x-ray crystallographer who could work a protein crystal the way Michael Jordan worked the basketball court. As much art as it is technique, x-ray crystallography provides a means of determining a protein’s atomic structure by analyzing the diffraction pattern produced by x-rays that are bounced off protein crystals. In his career, Steitz and various colleagues have been the first to decipher the atomic structure of many large biological molecules and molecular assemblies—in particular, those involved in converting DNA instructions into functional protein products.

### GETTING DOWN TO BUSINESS

Though microscopic, the ribosome is gigantic in molecular terms. The simplest ribosomes found in bacteria are perhaps 100 times larger than a typical protein. (Although they have some additional complexity, ribosomes of higher organisms are structurally similar to bacterial ribosomes.) Previous studies had established that the ribosome is an intricate tangle consisting of three strands of ribonucleic acid (RNA) and 54 proteins—by mass, about two-thirds RNA and one-third protein. Further, it consists of two subunits: a large subunit, called 50S, which makes up about two-thirds of the structure, and a small subunit, called 30S.

The Yale researchers focused on crystallizing the 50S subunit, working with ribosomes from *Haloarcula marismortui*, a salt-loving bacterium from the Dead Sea. Although ribosomes from *H. marismortui* had a reputation for being amenable to x-ray analysis, Steitz and his colleagues still faced a daunting technical challenge. “In fact, when we began, we weren’t even sure



MICHAEL MARSLAND

Members of the team that solved the ribosome structure: (from left) Nenad Ban, Thomas Steitz, Peter Moore and Poul Nissen. Not pictured is Jeffrey Hansen.

if the atomic structure *could* be determined,” Steitz recalls. “But not really knowing if this was possible made for some pretty interesting times during our lunches.”

The team began its project in 1995 and almost immediately ran into trouble. One problem the researchers encountered was “twinning.” When the researchers crystallized the 50S subunit, two mirror-image crystal structures formed within what seemed to be a single crystal. When the crystal was pulsed with x-rays, a confusing diffraction pattern emerged. “It took us two years to figure out how to overcome twinning,” Steitz says.

Unfazed by the setback, the group persevered, gathering most of its x-ray data at the National Synchrotron Light Source at Brookhaven National Laboratory on Long Island, New York. (“It helps to have a synchrotron just about in your backyard,” Steitz says.) The group also used the Advanced Photon Source at Argonne National Laboratory in Illinois.

Then came the calculations, “the fun part,” according to Steitz. The researchers used powerful computer programs to analyze the copious amounts of data collected—more than 6 million readings in one data set alone—and to produce maps showing the electron density of the atoms in the crystal. Next, they used

computer graphics software to “fit” three-dimensional atomic models developed from known chemical information into the electron density maps. *Fun?* “Fitting the models into the map is something like working a jigsaw puzzle, in that it involves a lot of pattern recognition,” Steitz explains. “In the end, we come away with a detailed picture, if you will, of the location of nearly all of the individual atoms that comprise the RNA and proteins in the ribosome crystal.”

The researchers methodically produced a series of structural maps of the 50S subunit at progressively finer resolution. In August 1999, they published in the journal *Science* a map of the 50S subunit at a resolution of five angstroms, or five ten-billionths of a meter, which is approaching atomic resolution. “This map gave us some important hints as to the ribosome’s detailed structure,” Steitz says, “but we needed to do even better in order to pin down the final answers.”

And so they did. In August 2000, the team published two reports in *Science* featuring the structure of the 50S subunit at a resolution of 2.4 angstroms. “At this resolution, we were able to resolve the atomic structure for nearly all of the 100,000 or so atoms in the crystal,” he says. “We can see that the approximately 3,000 nucleotides of RNA in the subunit

form a compact, complexly folded structure, and that the unit's 31 proteins permeate its RNA."

### THE RIBOSOME UNVEILED

The findings confirm much that was suspected about the ribosome's structure and function, but they also bring some surprises. "One important lesson we've learned is that the ribosome is actually a ribozyme—that is, an RNA machine," Steitz says. "The term 'ribozyme' was coined some years ago to signify a biocatalyst in which RNA performs the important work, as opposed to a conventional

enzyme in which proteins are the active materials. In the case of the ribosome, RNA catalyzes protein synthesis, and the proteins in the ribosome simply act as staples holding its structure together. There had been suggestions that this was the case, but there hadn't been direct proof. Now there is, beyond any doubt. The structure reveals that there are no proteins, only RNA, located anywhere near the active site where protein catalysis occurs."

The findings also shed light on the details of how ribosomes actually catalyze the construction of proteins. As might be

expected, the picture is exceedingly complex. In simplest terms, there are two parts to the process. In one part, the larger 50S subunit orients the transfer RNA (tRNA) molecules so that the relative positions of the newly delivered amino acid and of the growing peptide chain are optimal for forming bonds. This is similar to what conventional protein-based enzymes do—but in ribosomes the orientation occurs because of interactions between RNA components of the 50S subunit and the tRNA molecules rather than because of interactions between bits of protein, as happens with enzymes. In the

## CAUGHT IN THE ACT

Imagine going into a cell and watching the intricate writhing of a ribosome as it cranks out vital proteins. Structural biologists are nearing such powers of observation with new analytical techniques that manipulate ribosomes and other macromolecules while tracking changes in their structure and shape.

Among the most remarkable of the new methods is three-dimensional cryo-electron microscopy (cryo-EM). In the hands of researchers such as HHMI investigator Joachim Frank at Health Research Inc. at the Wadsworth Center in Albany, New York, cryo-EM has proven to be one of the few techniques capable of visualizing large, dynamic molecules. "Cryo-EM's strength is that it allows a three-dimensional reconstruction of macromolecules that cannot be crystallized for analysis by x-ray crystallography," Frank says. The technique also makes it easier and faster to explore how such dynamic molecules change

Here's how cryo-EM works. First, Frank and his colleagues immerse their macromolecules—let's say ribosomes—in a water solution. Then, they flash-freeze the molecules in supercold liquid ethane. The rapid freezing imprisons the ribosomes in ice, preserving their original conformation. Next, using an electron microscope with a low-intensity beam to avoid damaging the molecules, the scientists obtain images of thousands of the captive ribosomes. They then use sophisticated software called SPIDER, developed in Frank's laboratory, to transform these low-contrast, "noisy" images into a detailed, three-dimensional map of a ribosome.

Cryo-EM's power to detect the contortions of working macromolecules was highlighted this past July in a report by Frank and his colleagues in the journal *Nature*. The researchers announced that they had detected a subtle ratcheting rotation deep inside the ribosome—the complex biomolecule "powered" by ribonucleic acid—at a key stage in the protein-building process. In their paper, the scientists characterized the ratcheting as a rapid rotation of one of the ribosome's two subunits. This rotation occurs just as the messenger RNA (mRNA) and its two attached amino-acid-carrying transfer RNA (tRNA) molecules are advanced. Their analysis revealed that at a key point, the smaller subunit (called 30S) rotates about six degrees with respect to the larger subunit (50S). Then, after a chemical reaction advances the mRNA-tRNA complex, the smaller subunit rotates back. In this herky-jerky manner, the two subunits cooperate to build the long chains of proteins.

Discovery of the movement offers yet another clue to how the ribosome creates the enzymes and other components of the cell's machinery. "There have been hypotheses about subunit movement in the ribosome for years," Frank says, "but there has never been a direct confirmation of this. The problem was that all the evidence was indirect. And only now, with cryo-EM, can we visualize the ribosome with such clarity."

To Frank, the achievement underscores the broader promise of cryo-EM. "The niche for cryo-EM is likely to be pretty big," he says, "because it can be used to study macromolecular machines, like the ribosome, in all their different states, with all their necessary functional materials attached, as they normally operate in the cell." He emphasizes, however, that the far higher resolution achievable with x-ray crystallography makes the two analytical techniques partners rather than competitors. "It's not that x-ray crystallography puts cryo-EM out of business," he says. "On the contrary, it really puts it *into* business. By marrying these two techniques, one can proceed from the detailed atomic structure, from crystallography, to make sense of the different conformation you see with lower-resolution cryo-EM." —TB



CHRISTOPHER DENNEY

*The niche for cryo-electron microscopy is likely to be big, says Joachim Frank.*

shape as they carry out their biological functions. "In this way," he says, "we can essentially produce a movie of the macromolecule in action, which would be much more difficult and time-consuming to generate using other methods."

second part of the process, one of the ribosome's structural building blocks (specifically, the chemical base adenine) located at the site of protein synthesis has the unusual property of being just about neutral in its acidity. This condition helps promote the addition of each new amino acid onto the lengthening polypeptide chain.

Beyond helping to illuminate the innermost workings of cells, these findings hint at conditions that existed during the earliest days in the evolution of life. Since ribosomes in all classes of organisms share so many common features, many biologists believe that ribosomal structure has remained essentially unchanged over time.

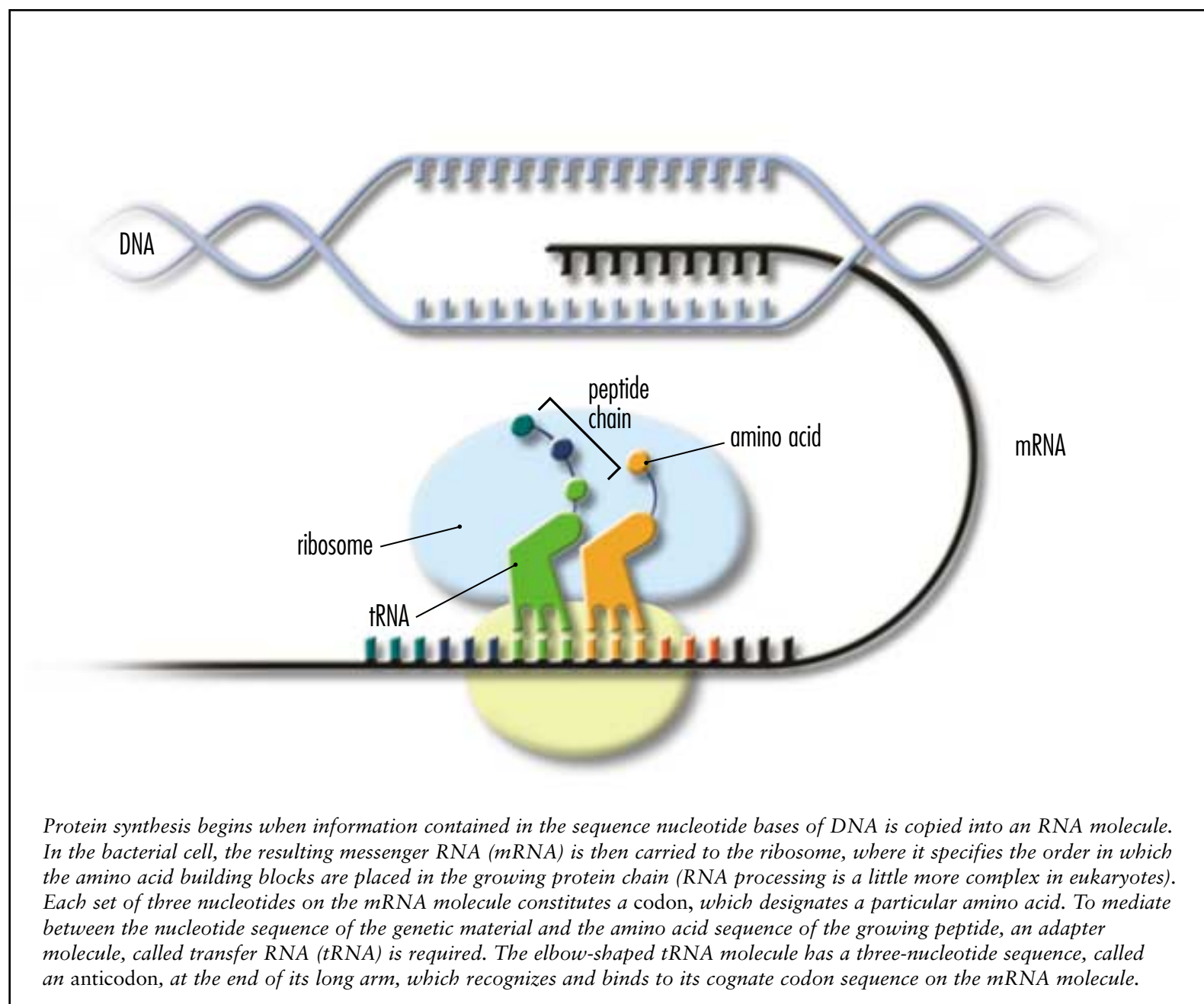
An increasing number of researchers argue that the first biocatalysts must have been RNAs rather than something resembling today's protein-based enzymes.

"It's the chicken-and-egg problem," Steitz says. "After all, before there were proteins, how could there have been a protein-catalyzed synthesis of a protein? Now, proving that ribosomes are indeed RNA machines adds considerable weight to the notion that, in the beginning, it was an RNA world, in which RNA was able both to convey genetic information and to catalyze important chemical reactions."

The new results may also point to a healthier future. Since every living cell needs machinery for protein synthesis, the

ribosome is one of the most important targets of antibiotics. With the detailed map of the ribosome, drug designers may begin to use an approach called structure-based design to develop new drugs that can effectively shut down the ribosomes of bacteria and other disease-causing organisms.

"Structure-based design can considerably speed up the drug-development process, since it provides researchers with a clearer picture of their structural targets," Steitz says. "Rather than having to develop thousands of chemical compounds at random and then test them for biological activity, as is typically the case today, researchers should be able to selectively design new compounds that



*Protein synthesis begins when information contained in the sequence nucleotide bases of DNA is copied into an RNA molecule. In the bacterial cell, the resulting messenger RNA (mRNA) is then carried to the ribosome, where it specifies the order in which the amino acid building blocks are placed in the growing protein chain (RNA processing is a little more complex in eukaryotes). Each set of three nucleotides on the mRNA molecule constitutes a codon, which designates a particular amino acid. To mediate between the nucleotide sequence of the genetic material and the amino acid sequence of the growing peptide, an adapter molecule, called transfer RNA (tRNA) is required. The elbow-shaped tRNA molecule has a three-nucleotide sequence, called an anticodon, at the end of its long arm, which recognizes and binds to its cognate codon sequence on the mRNA molecule.*

GEORGE EADE

are likely to be able to attack the ribosome.”

This approach may well help in dealing with today’s troubling spread of drug-resistant bacteria. “Many bacteria have developed resistance through mutations that have changed the ribosome’s shape,” Steitz says. “With the atomic structure of the ribosome now in hand, researchers should be better able to determine precisely where mutations have occurred and how such changes can be sidestepped by new drugs.”

Indeed, he says, the Yale team is “moving fairly quickly” into research on the interactions between the ribosome and various antibiotics. “We’ve been studying one particular family of common antibiotics, the macrolides, and we’ve identified a number of kinds of interactions that appear interesting from a medical standpoint.”

Such interests are even moving his group toward the world of business. “We’re now developing a small biotechnology company to take advantage of our knowledge about ribosome structure and our ability to explore how small organic molecules, which is what drugs are, bind to ribosomes,” Steitz says.

“By knowing the exact shape of the ribosome, we can design new chemicals tailored to fit into its various pockets and crevices. In a sense, we want to design ‘keys’ to fit into the ribosomal ‘lock,’ and this job obviously will be much easier if you know the shape of the lock. I’ve already had contact with lots of pharmaceutical companies, and we expect a number of them to join with us in trying to speed the development of effective new drugs,” he says.

On a more fundamental level, the team continues to study how the ribosome operates over time. “The atomic structure that we’ve determined represents the ribosome at only one state in its protein-building cycle. But the ribosome is a very complicated machine, and we need to understand its dynamics,” Steitz says. “Right now, it’s like trying to explain how a grandfather clock works just by determining the position of the pendulum at only one moment. This doesn’t reveal anything about what happens to the pendulum in the following moments, let alone about all the other gears and springs that are vital to the clock’s operation.”

Other teams have been making important advances in understanding the ribosome as well. This past September, a group led by Venki Ramakrishnan of the Medical Research Council in Cambridge, England, published an atomic-level structural map of the ribosomal small subunit, 30S. A team based in Israel and Germany and led by Ada Yonath, a pioneer in structural research on ribosomes, has published a similar, though less detailed, map of the 30S subunit. Harry Noller and colleagues at the University of California, Santa Cruz, have developed structural maps of the entire ribosome at a somewhat coarser resolution, with subunits joined together and some of the transfer RNA molecules attached. And Joachim Frank, an HHMI investigator at Health Research Inc. at the Wadsworth Center in Albany, New York, has used cryo-electron microscopy to reveal dynamic characteristics of the ribosome (*see page 15*).

Amidst this flow of information, however, the Yale team’s results are being heralded as a scientific milestone. “Just given the sheer immensity of the ribosome, this is a remarkable achievement,” says John Kuriyan, an HHMI investigator at The Rockefeller University. “It gives those of us working in structural biology hope that it will be possible to determine the structure and functions of other very large, very complex biological molecules—and in this business you are always looking for rays of hope. When I first read the reports of this work, I was filled with emotion on a very personal level. I felt much as I did when humans first stepped on the moon. I think this achievement will be just that important in the field of structural biology.”

Such praise “is nice, of course,” Steitz says, yet he remains firmly grounded. After years of puzzling over the structures of molecules, he has learned the importance of patience, of keeping things in perspective. “That’s why I took up gardening,” he laughs. “You prepare the soil, you plant the seeds, the flowers bloom and the flowers die—and you *still* haven’t completed the structures of the molecules you’re working on! So I use gardening as a way to get results that are more commensurate with the time frame of human emotions.”

## LURING RECRUITS TO STRUCTURAL BIOLOGY

Within the field of structural biology, the study of the interactions of large molecules or molecular assemblies is largely invisible to many of today’s graduate students, says HHMI investigator Joachim Frank.

“The students see the strong emphasis on molecular genetics and now the wave of bioinformatics, all of which concentrate on understanding genes and the structures of their protein products. What doesn’t get across is that some structural biologists are already, in a sense, beyond bioinformatics,” says Frank. “We are already working on understanding the dynamic interaction of the genetic products in the cell. In fact, we’re eventually going to develop three-dimensional reconstruction techniques for the cell.”

Despite such excitement, Frank says, he and other structural biologists often have difficulty attracting graduate students and postdoctoral researchers to the study of macromolecular interactions, and even some tenure-track positions in this area are going unfilled. Part of the problem, he believes, may be that new tools such as cryo-electron microscopy began to flower after the current crop of graduate students and younger scientists had already chosen their fields.

One solution, he suggests, is to begin educating potential structural biologists while they are still undergraduates. “For example, we’ve had great success at the Wadsworth Center in attracting students to the National Science Foundation Research Experiences for Undergraduates program,” Frank says. “We get about 800 applications, and we select the 20 best students to work with us.” In addition, Frank’s group employs promising high school students during their academic breaks. “We let the students assist in a variety of tasks, such as scanning micrographs and processing images, as a means of getting them interested in this field,” he says.

Grabbing students early seems to work. “We find that these young people are smart enough that after training, they immediately grasp the kinds of challenges we offer,” Frank says. “Their imaginations are fired and, as a result, they may be encouraged to look for graduate schools where they can pursue these types of studies in structural biology.” —TB