Large and complex, the ribosome has resisted efforts to decipher its structure for 4 decades, but now four groups have it in their sights, to the excitement of everyone in the field

The Race to the Ribosome Structure

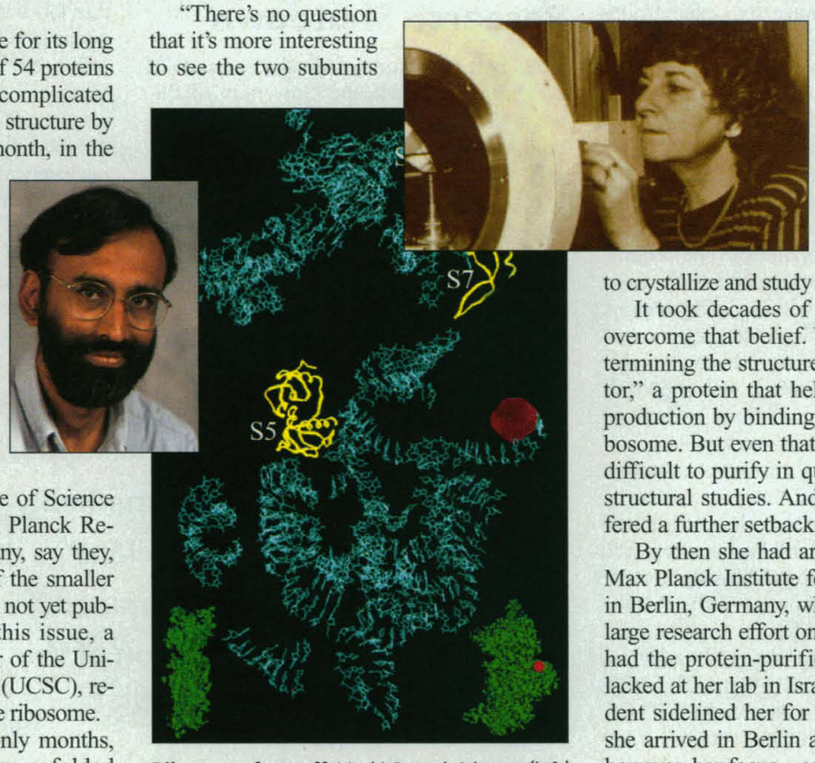
Every so often, an actress who has struggled through years of bit parts and waitressing gigs suddenly finds that she's an overnight success. The change comes when she lands that key role that finally shows off her talents. In the molecular world, a similar happy fate has now befallen the ribosome, the tiny particle in the cell that translates the genome's messages into all the proteins needed for life. After 4 decades in which researchers' best efforts to introduce the ribosome to a wider audience by laying bare its complete molecular structure have been frustrated, that structure is now having its debut.

The ribosome itself is to blame for its long period of obscurity. It's a tangle of 54 proteins and three RNA strands, which complicated every step needed to work out its structure by x-ray crystallography. But last month, in the 26 August issue of *Nature*, Venki Ramakrishnan's team at the University of Utah, Salt Lake City, published the structure of the smaller of the two subparticles that together make up a complete ribosome, while the team of Peter Moore and Thomas Steitz at Yale University described the structure of the larger subunit (*Science*, 27 August, p. 1343). Ada Yonath and her colleagues at the Weizmann Institute of Science in Rehovot, Israel, and the Max Planck Research Unit in Hamburg, Germany, say they, too, have solved the structure of the smaller subunit, although their results are not yet published. And on page 2095 of this issue, a fourth team, led by Harry Noller of the University of California, Santa Cruz (UCSC), reports the structure of the complete ribosome.

The structure's debut took only months, but behind the scenes the story unfolded over several decades, during which time Yonath doggedly worked to make progress. Only recently have others joined the fray, sparking what will be an intense sprint to the first truly high-resolution image of the ribosome and its subunits. The images are not quite there yet.

The resolution of the individual subunit structures, at between 4.5 and 5.5 angstroms, reveals the overall arrangement of the ribosomal proteins and RNAs, but does not show individual atoms. Noller's structure of the complete ribosome has a lower resolution, 7.8 angstroms, but does give the details of how the two subunits interact. What's more, the UCSC team also has three-dimensional images of the ribosome bound to messenger RNA (mRNA), which carries the genetic information needed for protein synthesis, and bound to transfer RNA (tRNA), the kind of molecule that supplies the ribosome with amino acids, the building blocks of proteins.

"There's no question that it's more interesting to see the two subunits together," says Moore. "If you want to see how protein synthesis occurs, the low-resolution map [of the whole ribosome] will be very significant." As Anders Liljas, a crystallographer at the University of Lund in Sweden, points out in his commentary on page 2077, ribosome researchers can now see the functional centers of the ribosome clearly. Thus, they can better check how their ideas on the mechanism of protein synthesis stack up against the real thing.



Ribosome face-off. Venki Ramakrishnan *(left)* published the first 30*S* subunit structure, but pioneer Ada Yonath *(right)* now also has the structure *(center)*, showing RNA (blue), two proteins (yellow), and mRNA (red).

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Breaking ground

Although three groups have gotten into print with their ribosome structures before Yonath, they and others credit her with paving the way. "She proved that it could be done," says structural biologist Wayne Hendrickson of Columbia University in New York City—something that many crystallographers doubted. When Yonath began her studies in the late 1970s, ribosomes seemed too big and too variable to crystallize and study by x-ray diffraction.

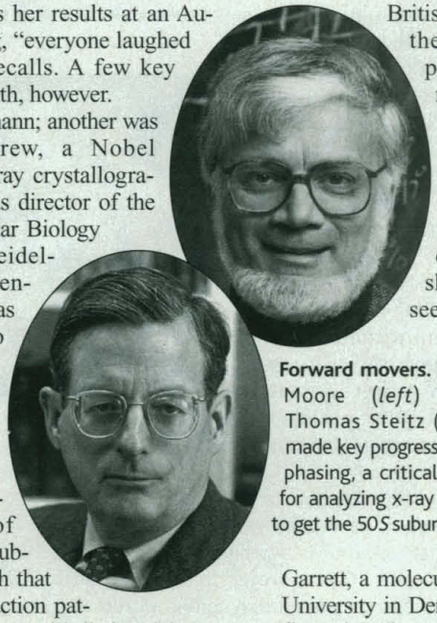
It took decades of persistence for her to overcome that belief. Yonath started by determining the structure of an "initiation factor," a protein that helps jump-start protein production by binding temporarily to the ribosome. But even that single protein proved difficult to purify in quantities sufficient for structural studies. And in 1978, Yonath suffered a further setback.

By then she had arranged to work at the Max Planck Institute for Molecular Genetics in Berlin, Germany, which had undertaken a large research effort on ribosomes and which had the protein-purification equipment she lacked at her lab in Israel. But a bicycle accident sidelined her for several months. Once she arrived in Berlin as a visiting professor, however, her focus—and her luck—changed. There she discovered a crystallographer's gold mine in the lab's refrigerators: lots of highly purified ribosomes left over from previous experiments. Yonath decided to ask the institute's director, Heinz Günter Wittmann, if she could try to crystallize them. "He said this was the dream of his life and gave me everything I needed," Yonath recalls.

Still, with the techniques then available, it took Yonath months of trying different so-

lutions and crystallization procedures to get tiny crystals of the larger, or 50*S*, subunit of the ribosome from a *Bacillus* bacterium, and more than a year to get the first very fuzzy x-ray crystallographic images. But when she showed colleagues her results at an August 1980 meeting, "everyone laughed at me," Yonath recalls. A few key people kept the faith, however.

One was Wittmann; another was Sir John Kendrew, a Nobel Prize–winning x-ray crystallographer who then was director of the European Molecular Biology Laboratory in Heidelberg. He helped ensure that Yonath was able to continue to get beamtime for her x-ray diffraction studies, despite the high risk of failure. Then in 1981, Yonath produced crystals of the *Bacillus* 50*S* subunit perfect enough that they yielded diffraction patterns in which she could distinguish atoms down to 3 angstroms apart from one another—an accomplishment that Hendrickson describes as "pivotal," because that resolution was about what would be needed to discern all the atoms in the structure.



Forward movers. Peter Moore (*left*) and Thomas Steitz (*top*) made key progress with phasing, a critical step for analyzing x-ray data, to get the 50*S* subunit.

Even so, getting to that resolution still proved difficult, because she needed stabler crystals that would last long enough in the x-rays for her to collect enough data to solve the ribosome's structure. Over the next several years she made a number of improvements. For one, ribosomes from the Dead Sea microbe *Haloarcula marismortui* proved stabler and better suited to x-ray studies than those from *Bacillus*. Together with Hakon Hope, a crystallographer at the University of California, Davis, Yonath made another key advance by adapting Hope's supercooling technique to the ribosome crystals so they would last longer in the beam of x-rays. "She was one of the first people to recognize that you had to cryocool [freeze] the crystals to get data," says Utah's Ramakrishnan. "She should get a lot of credit," now that the technique is universal in macromolecular crystallography.

With stabler crystals in hand, the next step was to figure out how to create specific landmarks for phasing in the diffraction patterns, a first step toward making sense of those patterns. Crystallographers typically do this by doping their crystals with heavy atoms, which contain so many electrons that they stand out like beacons on the electron-density maps. But because the ribosome is so big and electron-filled, Yonath needed larger concentrations of electrons than single atoms could provide and so decided to use clusters of heavy atoms instead. Even with these advances, by 1995 when the international ribosome community met in Victoria, British Columbia, her report on the 50*S* subunit was disappointing. And other ribosome researchers were beginning to champ at the bit.

Among crystallography's purists, the tradition has been that once a researcher crystallizes a challenging molecule, he or she is given the latitude to see that molecule through to its atomic resolution. After all those years, Yonath "had convinced everyone that you could do structural analysis [on the ribosome] by x-ray crystallography," recalls Roger Garrett, a molecular biologist at Copenhagen University in Denmark. But she hadn't really figured out how to solve the structure, and he adds, "People started getting impatient."

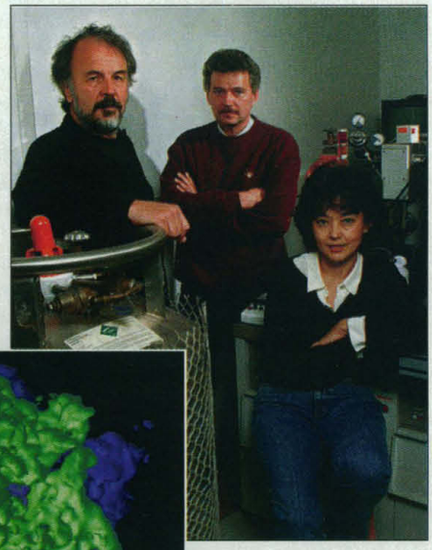
Parallel pursuits

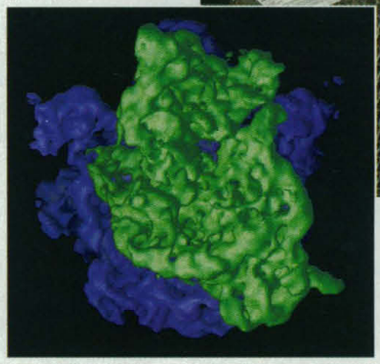
One rival effort had begun behind the Iron Curtain during the 1980s. Meeting as graduate students in Alexander Spirin's lab at the Protein Research Institute in Pushchino, Russia, Gulnara Zh. Yusupova and Marat Yusupov soon married and later embarked on their own pursuit of ribosome crystals at the institute in 1983. By 1987, they had produced crystals of the ribosome and of the smaller 30*S* subunit from the bacterium *Thermus thermophilus*. But Yusupov recalls, "It was impossible to solve the [structure] in Russia," because biologists there lacked access to the x-ray beamlines.

After the dissolution of the Soviet Union in 1989, however, the Yusupovs began collaborating with Dino Moras at the University of Strasbourg in France. There they produced a new crystal that diffracted better than the original one, but the work bogged down in the early 1990s because of shortages of funding. So in 1996, the Yusupovs packed up their crystals and moved to California to work with Noller at UCSC. "It was the right decision," Yusupov says.

Noller already had a 20-year interest in the ribosome, one that began when he sequenced and characterized its various RNA components. Because directly determining the entire structure seemed impossible at the time, he and his team developed biochemical approaches to figuring out where the RNAs link with the individual ribosomal proteins. But by the mid-1990s, Noller was ready to try the impossible. He recruited crystallographer Jamie Cate, who had just finished solving a much simpler RNA structure. "In terms of structural biology, this was the biggest challenge I could pick," Cate recalls.

At Santa Cruz, the Yusupovs improved their purification procedures, and Yusupova began working out ways to get crystals of ribosomes attached to pieces of either tRNA or mRNA. Gradually the crystals got better, and complexes of the ribosome with the other RNAs often proved stabler than the ribosome alone. Even as the crystals improved, the group was stumped about the best way to study them. At first it seemed "we just had to roll up our sleeves and fight with them," says Cate. Daily he and Yusupov would sit down for coffee to hash out ideas. Cate found Yusupov's methodical approach a good counter to his seat-of-the-pants, "let's do an experiment and work out the details later" impulsiveness, and the two were able to make progress on all fronts.





The full Monty. Harry Noller (left), Marat Yusupov (center), and Gulnara Yusupova (right) tackled the entire ribosome *(inset)*.

Noller's group also took an incremental approach to making sense of the diffraction patterns obtained from the team's crystals. As a first step, they used lower resolution structures reconstructed from images obtained by cryo-electron microscopy, in which speci-

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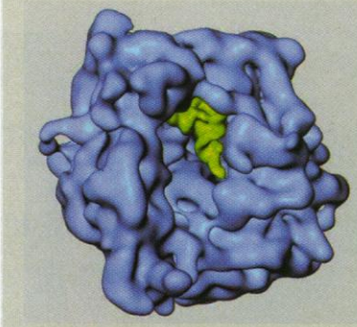
Challenge From Electron Microscopy

Physicist Joachim Frank may well feel a twinge of envy when he turns to pages 2095 and 2133 and sees the x-ray crystallographic images of the complete ribosome made by Harry Noller's team at the University of California, Santa Cruz. For 2 decades, both Frank's team and a rival group in Europe led by Marin van Heel have been pushing the limits of another imaging technology, called cryo-electron microscopy (cyro-EM), to achieve the same goal: seeing the ribosome—the cell's protein factory—in all its molecular detail. And 4 years ago, when both teams published structures resolved to about 25 angstroms, it seemed they might succeed ahead of the crystallographers. Now, crystallographers have taken the lead, but for understanding the dynamic nature of this molecular complex, cryo-EM is still very much in the running.

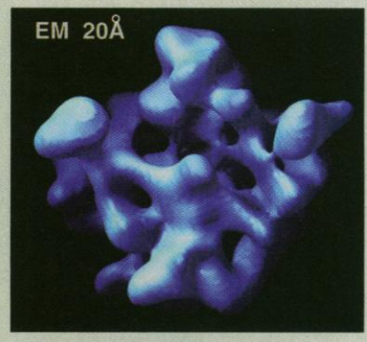
Before tackling the ribosome, Frank had spent years working out a computer program that could build two- and three-dimensional models of molecules by averaging and aligning electron micrographs of those molecules taken from different angles. He used the technique in 1981 to create a 2D reconstruction of the ribosome, but rapid progress didn't become possible until the late 1980s. By then, Jacques Dubochet of the European Molecular Biology Laboratory in Heidelberg, Germany, had developed a way of rapidly freezing molecules in water so that they end up encased in glassy vitreous ice, which lacks the crystals that would otherwise distort the structure.



Crystals unnecessary. This recent image of the *Escherichia coli* ribosome with transfer RNA (green) shows cryo-EM's potential.

Prepared this way, the ribosome could be seen much more clearly, and both Frank and van Heel, who was then at the Fritz Haber Institute of the Max Planck Society in Berlin, quickly applied cryo-EM to this structure. In 1991, Frank published the first 3D reconstruction of ribosomes based on cryo-EM. The resolution was only about 45 angstroms. But, Frank says, "we saw the two [ribosome] subunits and the triangular shape of the intrasubunit space."

Meanwhile, van Heel, who is now at Imperial College in London, was working out his own method for making 3D reconstructions. In 1995, at a Gordon Conference in New Hampshire, his group and Frank's "had two posters right next to each other," with models of the ribosome resolved to about 25 angstroms, van Heel recalls. For the first time, the molecule's surface topography, with all of its cavities and bulges, came into view. "All of a sudden, the ribosome had a spatial reality," Frank says. The researchers could see, for example, how the messenger RNA, which specifies a protein's composition, might thread itself through the ribosome. The images also helped crystallographers interpret their x-ray diffraction data. "Crystallographers wouldn't have gotten anywhere without the cryo-electron microscopy maps," says Roger Garrett, a molecular biologist at Copenhagen University in Denmark.



First structures. Early cryo-EM images of the ribosome's 50*S* subunit preceded good crystallography structures (opposite page).

Since then, both cryo-EM teams have been getting structures with progressively higher resolutions. Frank now has some 70,000 projections of the entire ribosome with an 11.5-angstrom resolution, and until recently, he says, "we thought we could win the race against Noller."

Van Heel still thinks he can win the next phase of the race: the quest for images with 4-angstrom resolution, almost good enough to reveal the positions of individual atoms. At the international ribosome meeting in Finland in June, he showed data on ribosome reconstructions with a resolution as fine as 8 angstroms. Frank isn't convinced that the resolution is that good, as he thinks the images didn't have the kind of detail he would have expected. But van Heel is bullish. "We're expecting a new microscope, and I'm expecting it to get below four [angstroms]," he says.

Even if the crystallographers also beat Frank and van Heel to the next milestone, cryo-EM has a unique advantage over crystallography. With cryo-EM, freezing a complex of molecules takes less than a second, instead of the weeks it takes to grow a crystal. So Frank and van Heel can easily catch the ribosome at various stages of operation, revealing how the molecules needed for protein synthesis move during the process. And that, notes Albert Dahlberg, a molecular biologist at Brown University in Providence, Rhode Island, "is going to be the next step, exploring the dynamic nature" of this protein factory. **–E.P.**

mens are frozen in vitreous ice before they are imaged (see sidebar). His team then refined those maps by diffracting x-rays of different wavelengths through ribosome crystals containing clusters of heavy atoms, a technique called multiwavelength anomalous dispersion. These clusters provided improved phasing information, which the researchers used to locate single heavy atoms inserted into another set of crystals.

This approach, aided by improvements in both the crystals and the synchrotron facility that provided the x-rays, helped the researchers surpass their expectations. "My feeling was that if [the crystals] only went to 12 to 15 angstroms, it would still be important," says Thomas Earnest, Noller's collaborator at Lawrence Berkeley National Laboratory in California. But so far the team has resolved the structure to 7.8 angstroms.

And they're off

Like Noller, Yale's Moore had been in the ribosome field for many years. With Yale colleague Donald Engelman, he had spent a decade working out the relative positions of all the proteins in the 30*S* subunit by scattering neutrons through crystals of the subunit, a structural technique that gives less precise information than x-ray crystallography. Still, says Noller, it was "a monumental piece of work." But as it began winding down in the early 1990s, Moore began to think seriously about the next step. "I got interested in higher resolution information," he recalls. For years, he and Yale structural biologist Steitz, who had distinguished himself by solving the structure of several proteins, had talked about tackling the ribosome.

In 1994, Steitz brought in Nenad Ban, who had worked on crystallizing viruses. Although Yonath's and Yusupov's work had shown that ribosomes can produce high-quality crystals, "we had to stretch every

CREDITS: (LEFT TO RIGHT) MALHOTRA *ET AL.*; FRANK *ET AL.*

available method to its limits," Ban says. The team decided to follow Yonath's lead, producing 50*S* crystals by using the methods that she reported as giving the best crystals. But like Yonath, they stumbled. "It was no wonder that she had trouble," says Moore. "These [crystals] are really hard."

The Yale team had particular trouble with "twinning," in which two mirror-image crystal structures form within what seems to be one crystal, resulting in confusing diffraction patterns. It took 2 years for the researchers to work out the problems, but once they were solved, "we were able to proceed very fast," Moore says. In the 26 June 1998 issue of *Cell*, they described a structure with 9-angstrom resolution, demonstrating that heavy atoms could be used to solve the ribosome structure. They then continued to improve on their data and analytical techniques, reaching 5-angstrom resolution by the spring of this year.

Meanwhile, Ramakrishnan, another longtime ribosome researcher, was edging toward the starting line as well. He had worked with Moore on the neutron scattering experiments from 1978 to 1982 and, while working first at Brookhaven National Laboratory on Long Island and then at the University of Utah, Salt Lake City, had helped solve the structures of about a half-dozen individual ribosomal proteins. He had become ever more eager to solve the structure of the 30*S* subunit, which plays the crucial role of accepting or rejecting the tRNAs that carry successive amino acids to be added to the protein.

He had also deduced from Yonath's presentation in Victoria that no one had really pursued getting good crystals of the 30*S* subunit, and he decided to try. His team managed to make steady progress, even though he moved across the Atlantic from Salt Lake City to the Medical Research Council Laboratory of Molecular Biology in Cambridge, U.K., earlier this year. During that time, though, Ramakrishnan never really let on that he was making this attempt. "He was the dark horse," says Joachim Frank, a physicist at the New York State Department of Health Wadsworth Center in Albany.

But when Ramakrishnan, Moore and Steitz, and Yonath gave consecutive talks about the structures they were working on at the opening session of an international ribosome meeting in Helsingør, Denmark, this June, Frank was most impressed by how far this dark horse had come. At the meeting, Ramakrishnan explained that turning his postdoctoral fellow Brian Wimberly loose on solving the structure "was like handing the keys of a Ferrari to a teenager." Wimberly not only sped picking out key landmarks in the resulting electron-density map, but also was able to trace parts of the RNA's path through the subunit.

Yonath also described her 30*S* research at that meeting, but her lower resolution results paled in comparison with those of Ramakrishnan and the others. Some of her colleagues, particularly her rivals, couldn't help but wonder if she had been left behind in the field she started. "She has invested most of her career in this problem," says Moore. "If she had been the first person to get the structure, there's no question that she would be given the Nobel Prize. Now, the final answer is unlikely to come from her."

While many in the audience were pleased to see new talent attacking the problem, a few crystallographers think that Moore and Steitz and Ramakrishnan should have held off. "She's done most of the backbreaking work," says Kenneth Holmes, a structural biologist at the Max Planck Institute for Medical Research in Heidelberg. "The others have jumped the gun. They should have left Ada in peace." But Yonath herself says she doesn't mind. "After being alone for so long, this gave the field a big push and gave me much satisfaction," she says. And colleagues who have seen her latest work, which she did not present at the June meeting, say she should not be counted out.

Indeed, everyone agrees that the race to the ribosome structure is not over. At the current level of resolution, says Steitz, "the [structure's] impact on biochemistry is small compared to what we want it to be." The ultimate goal is atomic resolution that reveals the exact interactions between the ribosome's components and thereby provides an understanding of how the structure makes protein synthesis possible. But like Yonath, he expects rapid progress. "We've defined for ourselves what's necessary to solve the structure," he says, adding that he expects the solution in a matter of months.

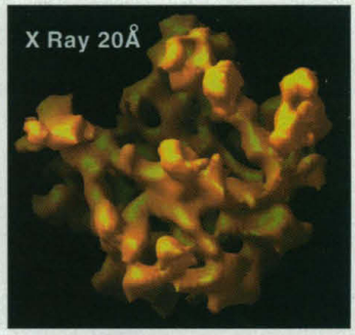
Yonath, for example, has already taken a step in that direction. In August at the XVIII Congress of the International Union of Crystallographers in Glasgow, U.K., she presented data on a 4.5-angstrom structure of the 30*S* subunit with, she says, "markers that mimic the functional sites." Liljas describes the structure as "very exciting," because the markers reveal where the mRNA hooks up with the subunit, presenting an even clearer picture of the ribosome than those now published.

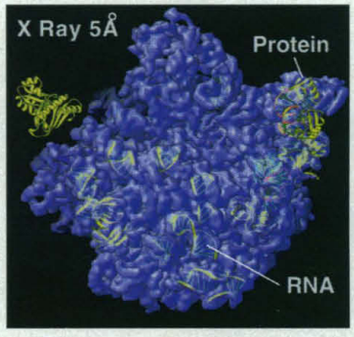
But the crystallographers point out that even the expected atomic resolution structure won't reveal everything about the workings of the ribosome during protein synthesis. The images from structural biologists "are snapshots," says Noller. The ribosome is really a machine that moves along the messenger RNA, all the while transferring amino acids from incoming tRNA and forging peptide bonds in the growing protein. To visualize all of this, says Noller, "what we really want is the movie."

That means capturing multiple images of the ribosome, recording each step. Making crystals suitable for x-ray diffraction studies of all those ribosome forms would be extremely difficult at best. It may be possible, however, with cryo-electron microscopy, which requires only that the various forms be properly frozen, although it's not clear that such studies will provide atomic resolution in three dimensions the way x-ray crystallography could.

But even if the "movie" turns out to be far in the future, researchers still expect benefits from the atomic resolution structure, and even from the less detailed structures like those that have now appeared. "There's a huge amount of biochemical evidence that can be put into perspective," says Ban. "It's going to be fantastic for everyone who is working with the ribosome."

–ELIZABETH PENNISI





Coming into focus. Gradually, details of the 50*S* subunit are coming into view as higher resolution structures become possible (top to bottom).

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