Piecing Together the Ribosome

For some, it's a lifelong puzzle

By ELIZABETH PENNISI

On a sunny August afternoon, Richard Brimacombe sits on a concrete wall, enjoying the sea breeze blowing off Long Island Sound. Moments earlier, this biochemist from the Max Planck Institute for Molecular Genetics in Berlin had dazzled an audience of molecular biologists with his computer rendition of the ribosome, the cell's protein-producing machine.

"I'm not a good scientist; I don't read the literature and things like that," he says by way of explaining why he works on solving the structure of the ribosome. "I need a problem that's like a crossword puzzle [so] I can sit in my little room and let the world go by."

Except the world won't go by. A senior scientist accosts him, feeling him out about moving to a different research institute. As that scientist leaves, two young researchers arrange for Brimacombe to send them data about where he thinks the various atoms in the ribosome's core are located. No sooner have they left than a woman from the translational control conference at the Cold Spring Harbor (N.Y.) Laboratory approaches. "Tell me, position 1,823 in ribosomal RNA — is it important?" she demands.

"Ah, 1,823, where is it? It's helix 66, about, isn't it?" he replies. He tries to recall where that one base — out of the 2,904 RNA building blocks he has plotted onto his computer model — is located. "Why do you ask?"

Hanna Engelberg-Kulka, a molecular biologist from Hebrew University-Hadassah Medical School in Jerusalem, hesitates. "It could be important. Is it exposed?" she asks. Brimacombe says he thinks so and offers to check a more detailed diagram so he can let her know for sure later. She leaves, and he shakes his head, perplexed by the attention.

These days, when most molecular biologists are caught up in the tidal wave of pinpointing and manipulating genes, the efforts of Engelberg-Kulka, Brimacombe, and others at the conference to understand RNA and ribosomes seem but a ripple. But ripples have a way of building into giant waves.

Even though genes encode all the information necessary for life, the information would go to waste were it not for RNA and ribosomes. Genes must realize their potential through molecules called proteins. Proteins — not genes — guide and carry out the chemical reactions necessary to make, fuel, and dispose of cells. Moreover, the genetic code may specify the amino acid building blocks of a protein, but it's up to the messenger RNA to get that information to the ribosome. Together, the messenger RNA and the ribosome read the code and make a protein.

"The ribosome presides over the translation of the genetic code," explains Harry F. Noller, a molecular biologist at the University of California, Santa Cruz. "It sits at the interface between the genotype and the phenotype." So, as ribosome researchers like to point out, the more genes scientists find, the more essential an understanding of this interface will become. That understanding will not come easy, they add.

With such an important job, it's no wonder that ribosomes are quite complex. Ribosomes have two subunits: a small one with several proteins and one strand of RNA, and a large one with proteins and two or three strands of RNA.

The RNA in each subunit folds and kinks, sometimes doubling up on itself, sometimes forming large loops. Various proteins wedge themselves into this tangled RNA. Then, in part because of this so-called secondary structure (the loops and kinks) and in part because of the influence of the proteins, the RNA becomes three-dimensional.

During the 1960s, researchers focused on the proteins in the ribosome as the key to protein production. "Most people thought the proteins did everything," says Noller. But by the 1980s, he and others had demonstrated that RNA plays a leading role, with ribosomal proteins part of an extensive supporting cast. These proteins seem to help RNA take on a particular three-dimensional configuration and may help modify that configuration, as needed, during ribosome assembly.

Determining that three-dimensional shape has been a goal — perhaps even an obsession — of Noller and Brimacombe. Both have pursued ribosomal structure in bacteria, pinning it down first in the small subunit.

Others studying RNA, ribosomes, and protein synthesis line up eagerly to take advantage of what these two men discover. They find the data useful for framing their own experiments and interpreting their own results. But Brimacombe and Noller are keenly aware of the limitations of their models. "They're useful as long as people don't take them too seriously," Brimacombe says. "It's nothing more than an attempt to get the best possible fit of the data at the stage you happen to be at."

"There are dangers that people will look at [a model] and say this *is* a ribosome," Noller says.

Noller and Brimacombe know better. Through the years, they both have had to revise and change their models. And they anticipate changes will continue for years to come.

Both researchers began decades ago by working out RNA's secondary structure — how each strand loops and folds back onto itself. Only certain bases will double up, so by comparing the sequence of nucleic acids in the RNA in ribosomes from many species, they were able to determine which nucleic acids pair off and which remain single. From that starting point, however, their paths have diverged, with each scientist arguing forcefully the value of his particular approach. Until the ribosome's atomic structure is actually determined, those differences will most likely remain unresolved.

"We started with what we think is the most accurate set of structural coordinates," Noller says — a neutron map of a ribosome's proteins published in 1987 by another research group. This map marks the position of each protein's center of mass. Noller and his colleagues used these centers as the basis of a three-dimensional connect-the-dots puzzle. The centers — the dots — served as a scaffold of sorts. By figuring out which proteins attached where on RNA, Noller's group could begin to thread RNA through the puzzle.

They tested how each protein "protects" the RNA by examining each one's effect under various conditions. They subjected each protein-RNA complex to enzymes that cut RNA into tiny pieces and to chemical probes, each of which modifies one of the four types of bases, or nucleic acids, that make up the RNA strand. "We prefer the probes because they are much smaller, so they can get into the nooks and crannies that an enzyme might not have access to," Noller explains.

His group compared their fragments and modified bases to fragments and modified bases in RNA with no proteins attached. Longer fragments indicated that a protein had prevented one of the enzymes from cutting the RNA in the usual place. Unmodified bases indicated that a protein had sheltered them from a

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chemical probe's attack. Taken together, the results revealed the "footprint" that each protein leaves on RNA. "We now have hundreds of these footprints," Noller says. He assumes that a protein shields the RNA "under" its footprint either by attaching to bases there or by somehow causing the RNA there to twist inward to avoid outside influences.

Each time Noller's group got a footprint, they placed its protein at the protected spot, gradually building a model of the RNA in the small ribosomal subunit. Then they fit these placements to the neutron map. In 1988, the group published its first three-dimensional picture of about two-thirds of the RNA in this subunit. They left out the rest of the RNA because their data did not provide enough clues about where to place it, he notes.

Next, they began using hydroxyl radicals as a chemical probe. These small, charged molecules provide a more detailed picture of how RNA threads through the proteins. The experiments enabled them to clear up ambiguous sections in the first model, notes Noller. A revision of this structure, still of the partial RNA strand, is about to be submitted for publication.

Noller says he's pleased that the new computer-generated model looks very similar to what electron microscopists see. The subunit forms a cleft. Most of the bases affected by a specialized RNA called transfer RNA cluster in that cleft. Each transfer RNA brings a particular amino acid to be assembled into proteins, so the cleft represents a key feature in the subunit.

"There are some paradoxes that we're still scratching our heads about," Noller cautions. For example, transfer RNA affects a section of ribosomal RNA called the 530 loop, yet Noller's map places that loop far from the cleft. To make matters worse, Brimacombe's data suggest that the loop may be near the cleft. "It's sort of a puzzler right now," he says.

Nevertheless, Noller stands firmly behind his approach, arguing that the neutron map should constrain the placement of RNA in any model. "It's our feeling that the neutron approach is more rigorous [than electron microscopy] because it's an accurate physical measure," he says. "Electron microscopy relies on interpretation — the different [research] groups get essentially the same picture, but the models they draw are different in details." Moreover, he cites the plethora of footprint data as helping to pinpoint each protein's place relative to the RNA.

It's possible, too, that both models are correct, he adds. At one point during protein production, RNA may kink to bring the 530 loop close to the cleft, while at other times the loop may drift far away. Noller may be modeling one step in this molecular dance and Brimacombe another.

"This is going to be a fascinating story for a long time to come," Noller adds.

Brimacombe's story also dates back many years and involves similar puzzles. He began by trying to figure out the location of each protein relative to the RNA in the small subunit. He and his colleagues put ribosomes into solution with an enzyme that chopped them into little bits, planning to study which proteins were attached to which bits of RNA. But once the ribosomes started breaking up, "all the proteins [started] hopping around and going completely random," he recalls.

So he added chemical connectors to solutions of the subunit and observed how particular proteins linked to specific parts of the ribosomal RNA. From this cross-linking data, he established the proteins' positions relative to the RNA.

By 1986, he had bent, crimped, and arranged 60 meters of wire (20 for the small subunit and 40 for the large subunit) into something that looked more suited to a museum of modern art than to a laboratory. Along with its turns and kinks, this construction contained short spirals of bases, or helices. There were 45 in just the 20 meters of the small subunit. When his group tried to publish a picture of this model, "the referees complained; they said it was impossible to see what was happening," Brimacombe recalls.

But computers were making their way into laboratories by then, so Brimacombe worked out a primitive computer model that depicted the helices as cylinders. It was published in 1988. He then decided that, since he had gotten this far, he might as well try to map all the atoms in all the nucleic acids of this RNA and link those atoms to the proteins' atoms. Getting a computer to put in that much detail would be no more difficult than simpler, more artificial representations, he reasoned.

Brimacombe decided not to let the neutron map guide him. "This is where my big difference with Harry Noller comes. He still uses that method, and I feel it's no longer the best way to do it," Brimacombe says. The neutron map pinpoints a protein's location in space but gives no information about the placement of that protein relative to the RNA, he notes. So when threading RNA through the proteins, it's anyone's guess whether it should be above, below, to the left, or to the right of a given protein. "It's a very floppy sort of placement," he explains.

Brimacombe favors working from the cross-linking data instead. He and his group have studied linkages not only between proteins and RNA but also between transfer RNA or messenger RNA and the RNA in the ribosome. In other experiments, they look at the connections between various ribosomal subunits. This approach "is making a lot of things that didn't fit together before fit together nicely now," he says.

(Bottom to top): A ribosome subunit depicted with wire, with computer-generated cylinders, and as helices with color-coded RNA sections. The uppermost image marks functional spots, including the locations of transfer RNA (large blue and red areas).

Brimacombe, Müller/Max Planck Inst.

Instead of bending wires, he and his colleagues are punching a keyboard. "We still have [the wire model], but it's gotten mutilated because I've been at it with the wire clippers," he says. Now, aided by Max Planck colleague Florian Müller, Brimacombe's group generates elaborate computer images. With a few keystrokes, they can reconfigure a section, leaving little trace of its former outlines. Moreover, they have harnessed the computer's power to compile all the data on the two subunits and have boldly portrayed the entire ribosome of a bacterium.

Not that this portrait doesn't have shortcomings, Brimacombe admits. "This [cross-linking] works very well when you are close to the messenger RNA or the transfer RNA, but the farther away you get from the functional center, the more garbagey it becomes," he says.

But he considers even the "garbage" worth pursuing. "It's a bootstrapping thing: You make the best thing you can and then somebody brings in new data. Either you say, 'Great, it fits!' or you say, 'Oh shoot, we've really screwed this up.' Hopefully, you gradually sort of get it."

The other attendees at the meeting think Brimacombe's on track. Several point to his talk as one of the meeting's most provocative and exciting.

Even so, Brimacombe downplays the significance of knowing where nucleic acid 1,823 is or of demonstrating that a cleft holds the binding sites for transfer RNA. "Basically, all we can say is something is near something else," he explains. "When you're asking questions of detailed function or how something works, one very rarely can say anything about it."

Nevertheless, he keeps refining the models. "I guess it's like anything else in science. You get your teeth into something and don't let it go." □