MOLECULAR BIOLOGY

Inseparable ribosomes

Fusing ribosomal subunits provides answers to basic questions about their function and opens the door to exciting engineering possibilities.

Ribosomes are the essential machines that translate mRNA into protein in all known organisms. Their design, consisting of independently associating small and large RNA subunits, is equally universal. Alexander Mankin from the University of Chicago, in collaboration with Michael Jewett from Northwestern University, wanted to know why a ribosome needs two separate subunits. Is it because they descend from two different enzymes, or is this design the only one that can lead to a functioning ribosome?

The researchers were also interested in creating an orthogonal ribosome, one that would translate solely an mRNA of choice and leave the rest of the protein translation to the wild-type ribosome. "People have been trying to establish an orthogonal translation system to expand the scope of what a ribosome can do," says Mankin. But because the two subunits associate randomly, it was impossible to have a fully orthogonal ribosome.

During translation, the small subunit first binds to the Shine-Dalgarno sequence on the mRNA. When it finds a start codon, the larger subunit associates and catalyzes the transfer of amino acids to the growing peptide chain. When the ribosome encounters a stop codon, it dissociates from the mRNA and releases the protein. Although it is possible to engineer a small subunit that associates only with a target mRNA, modifying the large subunit can be detrimental for the cell. If, for example, it is designed to accommodate unnatural amino acids, the modified large subunit will at some point also associate with a wild-type small subunit and not function for normal translation, leading to cell death.

This random association needed to be prevented. "We wanted to make a tethered ribosome, so we can have an orthogonal small subunit [that] is handcuffed to its own larger subunit," explains Mankin.

Cedric Orelle, who was a postdoctoral fellow with Mankin at the start of the project in 2012, and Erik Carlson, a graduate student with Jewett, took on the task of linking the two subunits. The researchers took advantage of the fact that ribosomal RNA in the large subunit is essentially a circle in which the 5′ end base-pairs with its 3′ counterpart. They joined the natural ends together and introduced new ends, which they fused to the RNA of the small subunit at the interface of the two subunits. Among several different constructs, they found one that worked. "No one could predict which design would work. The chances of any working were low; we were lucky," concluded Mankin, while commending Orelle and Carlson for taking on such a high-risk project.

The tethered ribosome, RiboT, could support normal translation in bacterial cells that had no wild-type ribosomal RNA. For Mankin, this settles the basic biology question. "Now we have a strong argument that the ribosome is built of two subunits not because it has to be but because it evolved like this," he says. "It likely descends from two RNA enzymes."

RiboT also opens the door to a range of applications: the subunits can be engineered to translate proteins that are problematic for a wild-type ribosome, and they can accommodate unnatural amino acids. In the near future, Mankin and Jewett want to focus on improving RiboT to increase its efficiency, which is currently about 50% that of a normal ribosome.

And "if you start dreaming," as Mankin puts it, one can envisage designing RNA-only ribosomes that function without their essential proteins, making their manipulation easier. Or one could even create tethered ribosomes that polymerize substrates other than amino acids in a programmed way. Mankin's prediction: "probably not today, maybe not tomorrow, but the day after tomorrow."

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RESEARCH PAPERS

Orelle, C. *et al.* Protein synthesis by ribosomes with tethered subunits. *Nature* **524**, 119–124 (2015).