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High-Resolution Image Illuminates Catalytic Engine of the Ribosome

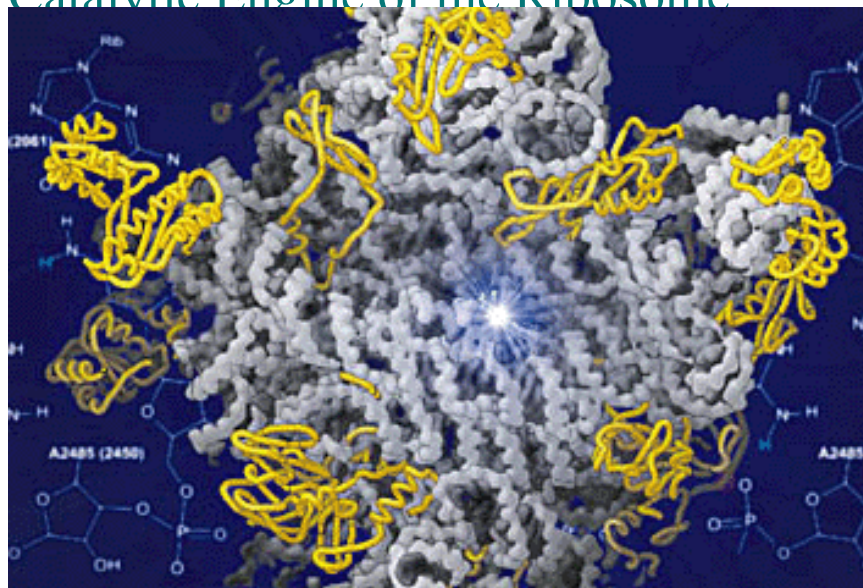


Image Title: The image in the foreground shows the RNA/protein architecture of the large ribosomal subunit with the active site highlighted. The background shows a schematic diagram of the peptidyl transferase active site of the ribosome. - Nenad Ban, Poul Nissen, Jeffrey Hansen, Peter B. Moore, Thomas A. Steitz

Using a high-energy x-ray beam to probe fragile crystals of RNA and protein, researchers have obtained the most detailed images ever produced of the cellular factory where amino acids are linked into chainlike proteins.

The studies illuminate the basic structure of the ribosome, a protein-making machine found in all cells. These insights include the first unequivocal proof that the ribosome is a ribozyme, an RNA enzyme.

In two articles published in the August 11, 2000, *Science*, researchers led by Thomas A. Steitz a Howard Hughes Medical Institute investigator at Yale University, report that they have obtained the atomic structure of the 50S subunit of the ribosome at a resolution of 2.4 angströms. An angström is one ten-billionth (10^{-10}) of a meter.

The ribosome is a large molecular complex of RNA and protein. When ribosomes are isolated from cell extracts, two different fractions are obtained, representing two subunits. The smaller 30S subunit binds the messenger RNA that constitutes the protein's genetic blueprint, as well as the transfer RNA that carries each specific amino acid to be added to the growing chainlike protein molecule. The larger 50S subunit catalyzes the formation of the bond between each amino acid and the growing protein chain.

Steitz and his colleagues at Yale University used the 2.5 billion electron volt x-ray beam at Brookhaven National Laboratory's National Synchrotron Light Source to perform x-ray crystallography on crystals of 50S subunits that were produced with osmium and iridium atoms attached to act as landmarks. Additional data were gathered using the Advanced Photon Source at Argonne National Laboratory.

In x-ray crystallography, protein crystals are bombarded with intense x-ray beams. As the x-rays pass through and bounce off of atoms in the crystal, they leave a diffraction pattern, which can then be analyzed to determine the three-dimensional shape of the protein.

"Our previous maps of the 50S subunit at nine- and five-Ångström resolution gave us some hints at the structure, but not until we achieved the 2.5-Ångström resolution could we resolve the atomic structure of all 100,000 atoms that are well ordered in the crystal," said Steitz. "This structure is about four times larger than any other such structure that has ever been determined, and the 3,000 nucleotides of RNA increased the amount of known RNA structure by about 4 to 5 fold."

According to Steitz, the process of achieving such high resolution meant painstakingly improving the process of growing larger, more complete ribosome crystals, and solving structures of those crystals at progressively higher resolution. Each lower-resolution map provided information that could help the scientists understand the ultimate high-resolution map, he said.

"I think we were amazed at each stage at the overwhelming complexity of the RNA folding in the ribosome," said Steitz. "But I think the most surprising observation was that the proteins were embedded among the RNA helices, penetrating into the interior of the ribosome like tentacles."

Such penetration of proteins explains why previous researchers had not been able to show that the ribosome depended solely on RNA as its catalytic molecule," said Steitz.

"Since (HHMI President) Thomas Cech had shown that RNA could have catalytic activity, we had suspected that the 50S subunit was basically a ribozyme," said Steitz. "However, there was no proof. Nobody had been able to show that the RNA by itself showed catalytic properties in the absence of the protein. Now we can see that part of the reason is probably the nature of these proteins that are holding the ribosome together.

"Our structure shows that these proteins are deeply embedded in the RNA and are essential for its folding. And it shows unambiguously that the ribosome is a ribozyme because we can see where the substrate binds and there's no protein atom near enough to that site to produce any catalytic activity."

The structure also provides intriguing insights into how the ribosome might originally have evolved, perhaps as a machine to make short proteins, or peptides, said Steitz.

"Earlier experiments by Cech and others had shown that it was possible to create RNA molecules that have some of the catalytic properties of the ribosome in peptide synthesis," he said. "Now we can see in this structure that some aspects of the native ribosome reflect some aspects of those RNA molecules produced through *in vitro* evolution. So, the expectation that a small RNA molecule could have evolved to catalyze peptide bond synthesis is not a far stretch.

"However, that peptide-making RNA molecule would not have been directed by messages from some early genome," he added. "How evolution managed to progress from making a random peptide to messenger-directed synthesis, we haven't a clue."

According to Steitz, the latest high-resolution structure offers a pathway to far deeper understanding of the protein-assembling machinery. The researchers are planning further studies to understand how the messenger RNA and components of the growing protein are oriented in the ribosome's catalytic active site. They will also explore how the ribosome structure influences the chemical properties of the molecular groups in the active site. And, the scientists will seek to understand how the multitude of magnesium and potassium ions and water molecules integrate into the ribosome and stabilize it.

"We're certainly not done with the scientific challenges presented by the ribosome," said Steitz. "Although I must say I do feel as if we're standing on Mount Everest at the moment and I'm now looking to find K2."