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Complexities of the Eukaryotic Ribosome Revealed in New Structural Model

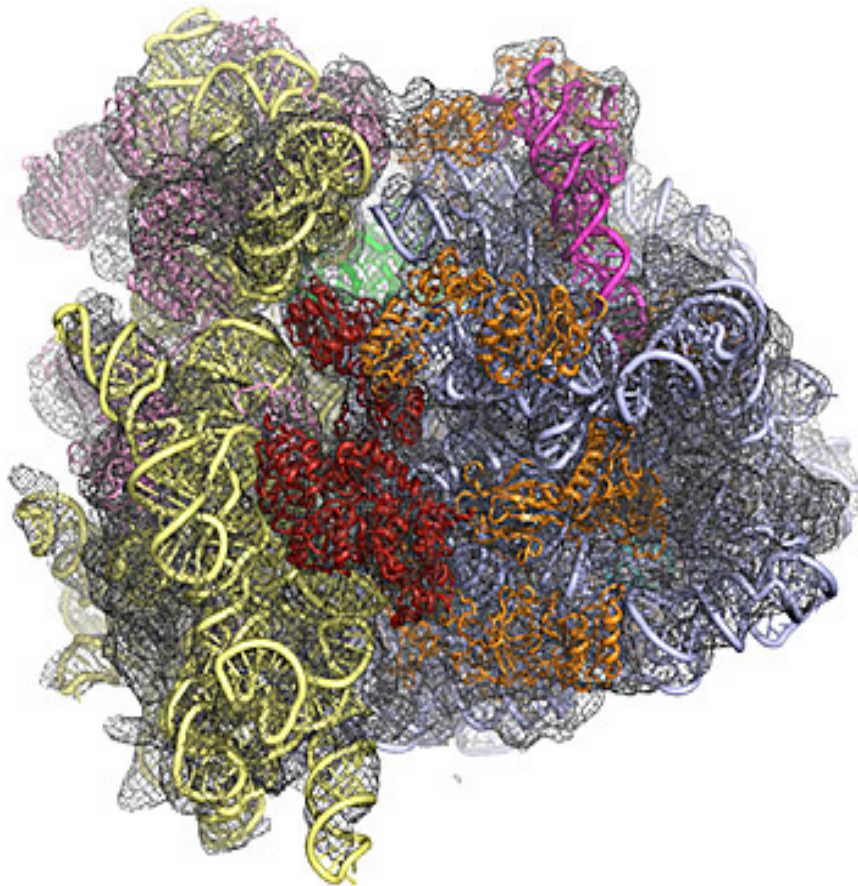


Image Title: Cryo-EM density map (mesh) and fitted atomic structure for the eukaryotic ribosome. The elongation factor dEF2 is depicted in red. - Joachim Frank, *Structure*, Dec. 1, 2009

Using advanced electron-microscopy and modeling techniques, a team of researchers has determined the structure of a eukaryotic ribosome with unprecedented accuracy. Although bacterial ribosomes have been described

in great detail, biologists had known much less about the larger, more complex ribosomes of higher, multicellular organisms (eukaryotes).

The primary function of a ribosome is to translate the genetic information encoded in messenger RNA into functional proteins. Life depends on this process, and scientists have long sought to thoroughly understand the ribosome's structure and function. Piecing together the precise molecular structure of the ribosome has required decades of biological detective work by multiple laboratories.

"This is an accomplishment that really makes a lot of other work possible," said Joachim Frank, a Howard Hughes Medical Institute (HHMI) investigator and structural biologist at Columbia University. "With this working model we can now understand and predict more of the eukaryotic ribosome's biochemical interactions with other molecules in the cell. It is a first step toward determining its atomic structure." Frank and his colleagues published their work in the December 9, 2009, issue of the journal *Structure*.

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Aided by other proteins in the cell, ribosomes assemble new proteins according to genetic instructions. They use messenger RNA as a blueprint as they string together amino acids, the building blocks of proteins, in a defined sequence. To add a single amino acid, the ribosome must first screen nearby transfer RNA molecules until they find one carrying the amino acid specified by the RNA code. Once that amino acid is added to the growing protein, the messenger RNA and transfer RNA, temporarily paired, are moved through the ribosome so that the next piece of the code can be read and the next amino acid incorporated.

Even in the simplest organisms, ribosomes are made up of dozens of proteins and strands of RNA. It was only a decade ago that the structures of some bacterial ribosome species were solved at atomic resolution. That work was recognized with the 2009 Nobel Prize for Chemistry, which will be awarded this week to three leading ribosome researchers, including HHMI investigator Thomas A. Steitz, Ada Yonath of the Weizmann Institute of Science in Israel, and Venkatraman Ramakrishnan of the MRC Laboratory of Molecular Biology in England.

The challenge since then has been to determine the atomic structures of the larger, more complex ribosome of eukaryotes. Nearly 80 proteins and four

RNA molecules come together to form each protein-making machine inside eukaryotic cells. Initial structural models of these complex molecules were low in resolution and contained too many gaps to be very useful.

The central core of the ribosome, which is responsible for protein synthesis, is quite similar across species, since even a subtle alteration here can affect the ribosome's ability to synthesize proteins properly. That can have far-reaching effects on an organism's ability to survive and reproduce, and thus the ribosome's core has changed little as organisms evolved. The periphery of the eukaryotic ribosome, however, contains many proteins and RNA segments that are not found in bacterial ribosomes. These vary between species and their functions are largely unknown -- but most appear to be necessary for cells to survive.

A big leap forward in understanding how the eukaryotic ribosome is composed required collaboration between several labs. Frank's lab led the imaging effort, using cryo-electron microscopy (cryo-EM), in which samples are flash-frozen in a thin film of ice before being imaged with a beam of electrons. The technique uses fewer electrons than in traditional electron microscopy, and therefore damages samples far less. But the low electron "dose" also means that the images are largely obscured by random electronic background noise. "The noise is so extraordinarily high that we need to use very sophisticated methods to extract the signal," said Frank.

Frank's team created obtained a hundred thousand separate images of ribosomes from a fungus closely related to yeast -- supplied by collaborator Poul Nissen at the University of Aarhus in Denmark -- and used computer algorithms to pull a three-dimensional image out of the noise. The high-resolution image they produced revealed how a protein called eEF2 binds to the ribosome to trigger the move of messenger RNA and transfer RNAs during protein synthesis.

That success motivated Frank and his postdoctoral fellow, Derek J. Taylor, then at the New York State Department of Health's Wadsworth Center, to seek out experts in computer modeling of RNA and protein for further studies. building a model consistent with the three-dimensional ribosome map and existing structural and sequence knowledge. Steve Harvey, a specialist in RNA modeling at the Georgia Institute of Technology, used what was known about the structure of the RNA component of the bacterial ribosome to make predictions about the RNA structure in the yeast ribosome. Comparing the sequence of the RNA in the different species, Harvey could identify segments of RNA in the yeast ribosome that were not present in the simpler ribosome, then fit these into the cryo-EM-based model from Frank's lab.

Andrej Sali at the University of California, San Francisco, filled in the gaps on the eukaryote ribosome map using protein homology modeling. From analyses of the material in yeast ribosomes, Sali and his colleagues knew the molecular sequences of their proteins. So they sought closely matched, evolutionarily-related sequences in the bacterial ribosome, for which good

structural information was available. Where these matching sequences could be found, Sali and his colleagues could infer the likely structures of the eukaryote versions.

Once these steps were complete, Taylor combined the data into the final model of the yeast ribosome. The new model includes more than 50 new segments not found in bacterial ribosomes, including what appear to be key binding areas for other proteins that interact with ribosomes.

“It serves as a reasonably detailed working model. We now can start to predict interactions of the eukaryotic ribosome with outside factors that are important to the regulation of its functions,” Frank said. “The new information will also be pivotal in establishing molecular differences between eukaryotic and bacterial ribosomes.” This could help researchers design antibiotics that shut off protein synthesis in bacteria without affecting the host’s ribosomes, he said.

Frank and his colleagues are now working to fill in the remaining gaps in the model and improve the structural imaging resolution to produce a final, atomic-scale model. The current paper represents a big step towards that complete structure, which is expected to have major implications for disease research as well as basic cell biology. Frank hopes some newly-introduced improvements to cryo-EM, which enable better image contrast and more efficient detection of the imaging beam, will help his team reach the goal more quickly. Even then, he said, “the data collection is going to require a gigantic effort.”