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Technique Captures New Information about Protein Synthesis Machinery

Howard Hughes Medical Institute researchers have deduced the structure of a molecule that orchestrates the early stages of protein synthesis. The scientists have used the newly solved structure to better understand protein synthesis and to learn how the hepatitis C virus might hijack the protein synthesizing machinery in human cells.

The new view of the protein complex, known as eIF3, reveals a five-lobed structure, with the lobes arranged much like a head, arms, and legs. The scientists' findings help explain how eIF3 uses these appendage-like lobes to maneuver components of a cell's protein-making factory, allowing the conversion of RNA to protein to begin. Their findings also reveal how hepatitis C virus (HCV) interacts with eIF3, a finding that could yield new drug targets to treat HCV, they said.

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— Eva Nogales

The research involved a collaboration between the laboratories of HHMI investigators Eva Nogales and Jennifer A. Doudna, both at the University of California, Berkeley. Bunpote Siridechadilok and Christopher S. Fraser were co-lead authors of the research paper, which was published in an early online article in *Science Express* on December 1, 2005. Another co-author, Richard Hall, is at Lawrence Berkeley National Laboratory.

The genetic blueprint contained in messenger RNA molecules (mRNA) is translated into protein by the cells' ribosomes. In order for protein synthesis to begin, two major components of the ribosome, known as the 40S and 60S subunits, must come together to form one large complex. Proper initiation of this process requires eIF3.

According to Nogales, prior to this study little was known about how eIF3 worked. Researchers did know that eIF3 prevents the two components of the ribosome from assembling prematurely by binding to the smaller of these, the

40S subunit. Previous studies by Doudna and HHMI investigator Joachim Frank at the Wadsworth Center revealed that HCV and other viruses commandeer this protein machinery by insinuating their own RNA into the ribosome using an internal ribosome entry site (IRES).

“However, the structure of eIF3 had not been studied by itself,” said Nogales. “And although we had some idea of where eIF3 would bind to the ribosome, there was no direct information on its structural organization. Nor was there any information on how it interacted with the viral IRES. What is really novel in this work is that we analyzed the structure of human eIF3 and its interactions with the cap-binding initiation complex mRNA and the ribosome, as well as eIF3's interaction with IRES,” she said.

The researchers analyzed eIF3's structure using cryo-electron microscopy, a technique in which the large protein complexes are suspended in water solution and plunged into liquid ethane. Freezing imprisons the protein particles in their native state in hardened vitrified water. Using an electron microscope with a low-intensity beam to avoid damaging the molecules, the scientists imaged thousands of the captive particles. The scientists then used computerized image analysis to produce a three-dimensional map of the complex from the low-contrast, noisy images produced by the electron microscope.

According to Doudna, the three-dimensional structure of eIF3 was distinctive. “This three-dimensional reconstruction revealed five well-defined lobes that for the sake of convenience, we named the head, arms and legs,” said Doudna. Using this new structural knowledge, the researchers began to model how eIF3 interacts with the 40S ribosome subunit.

In particular, Nogales and her colleagues modeled how the viral IRES could interact with the eIF3 complex. “Our studies had shown that the IRES was flexible, and although it was grabbing onto the eIF3, it was changing conformation, like a handkerchief flapping in the breeze. But we found one particular conformation, which was the most abundant, that the IRES had previously been shown to take when it was bound to the ribosome.”

However, said Nogales, the most striking findings revealed how both cellular mRNA and viral RNA interact with eIF3. “The most important insight our collaboration yielded was that eIF3 functions the same in both protein synthesis pathways—the one the virus uses and the one the eukaryotic cell normally uses to place the mRNA in the right place within the translational machinery,” she said.

Doudna added that the structural studies yielded telling insights into how eIF3 manages ribosomal assembly. For example, the scientists found that the “left toe” of eIF3 covers a segment of the 40S subunit that may prevent premature binding with the other component of the ribosome, the 60S subunit.

The findings could have important implications for understanding and treating HCV infections. “We now have a better mechanistic understanding

of why this hepatitis virus IRES RNA can take over the protein synthesizing machinery and get around normal controls,” said Doudna. “My hope would be that in the future we could find either small molecules or mutants of some of these proteins that would allow us to interfere with that interaction between the HCV IRES and the translational machinery.

“We don't have the molecular resolution yet to see how these interactions work and how to design drugs to block them,” Doudna said. “Nonetheless, it's important and helpful to know where these interactions occur, and we are pushing for higher resolution so we can begin to find such targets.”