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## Hepatitis C Virus Clamps onto Protein Synthesis Machinery

Researchers have discovered that the hepatitis C virus (HCV) employs an unusual strategy to induce a host cell's protein-making machinery to synthesize viral proteins. The research could provide a promising target for the development of new drugs to block HCV infection without harming body tissues. According to the scientists, the studies also suggest new details about how messenger RNA (mRNA) induces the initiation of protein synthesis.

The findings by a research team led by Howard Hughes Medical Institute (HHMI) investigators Jennifer A. Doudna and Joachim Frank were reported in an article in the March 9, 2001, issue of the journal *Science*. Doudna and her colleagues are at Yale University and Frank and his colleagues are at Health Research Inc., at the Wadsworth Center in Albany, New York.

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Existing drug therapy for hepatitis C often fails, so researchers have been looking for new molecular targets for HCV therapy. In the United States alone, 10,000 people die annually due to HCV infection. The death toll from HCV is so high because the majority of people infected with HCV develop chronic liver disease, cirrhosis, or liver cancer. The Centers for Disease Control and Prevention estimates that in the United States, HCV infection is responsible for \$600 million a year in health care costs and lost wages.

In their study, Doudna, Frank and their colleagues sought to determine how HCV, as well as other viruses, uses an alternate protein synthesis initiation method involving internal ribosome entry sites (IRES) to insinuate its messenger RNA into the principal component of the protein-making machinery, the ribosome. Ribosomes are large globular protein-RNA "factories" that read mRNA and translate its genetic information into proteins.

IRESes are structured sequences of viral mRNA that, in essence, commandeer the protein synthesis machinery, diverting it from its normal job of making cellular proteins. Protein synthesis is normally initiated when the ribosome recognizes a characteristic nucleotide cap on the end of the cell's mRNA. "It's been known for many years now that a number of viruses use this internal mechanism of initiating protein synthesis," said Doudna. "It's actually quite a nifty evolutionary trick because it allows the virus to shut down host protein synthesis, while continuing to pump out viral proteins.

"However, what's also interesting about these internal ribosome entry sites is that some host cell proteins also seem to use this mechanism for making certain types of protein. And these host proteins seem to be involved in the central control of transcription or other very fundamental cell functions. So, the theory is that cells also use IRESes as a way of expressing these important proteins under times of duress or viral infection, or when other protein synthesis is being shut off," Doudna said.

The scientists probed how HCV utilizes its IRES by using cryo-electron microscopy (cryo-EM) to create high-resolution three-dimensional maps of the viral IRES RNA bound to a ribosome subunit, called the 40S subunit.

Three-dimensional cryo-EM is one of the few techniques capable of visualizing large, dynamic molecules. In preparing for cryo-EM, researchers first immerse the IRES-ribosome subunit particles in water solution and then abruptly freeze them in supercold liquid ethane. The rapid freezing imprisons the bound complexes in ice, thus preserving the particles' native structure. Using an electron microscope with a low-intensity beam to avoid damaging the molecules, the scientists obtained images of thousands of captive particles. The scientists then employed sophisticated computerized image analysis to produce a detailed, three-dimensional map of the particle complexes from the otherwise low-contrast, noisy images produced by the electron microscope.

"This was a fairly straightforward application of cryo-EM," said Frank. "The only problem we had was that many of the ribosomal particles were not occupied with IRES RNA, so we had to collect data on more particles to get a useful map. However, cryo-EM was clearly the only way these maps could have been made, since crystallizing such a large complex for analysis by x-ray crystallography is simply not feasible."

Cryo-EM maps of the 40S subunit revealed that it consists of three domains, called the "body," "head" and "platform." To identify which segment of the viral IRES bound to the ribosome subunit, the scientists first produced maps of the subunit complexed with a truncated IRES that lacked a segment called "domain II." They found that deletion of this domain did not affect binding to the ribosome, although the truncated IRES could not efficiently initiate protein synthesis. Next, the scientists produced maps of the normal, or "wild-type" IRES complexed with the ribosome subunit. These new maps revealed the likely function of the fingerlike domain II region of the viral mRNA.

"When we compared the maps of truncated IRES to the wild-type IRES and to the empty ribosome as well we found what appears to be quite a large conformational change that's induced in the ribosome by the wild-type IRES," said Doudna.

"It looks as if the IRES is pushing on the head of the ribosome and causing it to reach down and fuse with the platform and the shoulder region," she said. "In effect what this does is to turn the cleft where we think the messenger RNA normally binds into a tunnel. So, the IRES is basically clamping the ribosome around the messenger RNA."

According to Doudna, the likely effect of the clamping is that it locks the viral messenger RNA into the correct site on the ribosome, so that the ribosome is forced to initiate production of a viral protein.

"This is the first time any kind of initiation complex with the eukaryotic ribosome has been visualized," said Doudna. "One very exciting possibility is that this conformational change might turn out to be a general way that the translational machinery is manipulated, even with the more common cap-dependent initiation mechanism."

However, she said, the universality and detailed function of the protein-synthesis-initiating conformational change will be determined only after further experiments and analysis of higher-resolution maps. Doudna also said that additional studies would be needed to determine whether drugs that block the IRES mechanism could serve as an effective treatment for HCV.